ANNUAL TECHNICAL REPORT

2062/063 (2005/2006)

Government of Nepal
Ministry of Agriculture & Cooperatives
Department of Livestock Services
Directorate of Animal Health
Central Veterinary Laboratory
Tripureshwor, Kathmandu
Phone : 4261938, 4212143, 4261165
Fax : 4261867
E-mail : cvl@wlink.com.np

ANNUAL TECHNICAL REPORT

F. Y. 2062/063

[2005-2006]

Editorial Board

Dr. Rebati Man Shrestha
Chief Veterinary Officer, CVL

Dr. Ganesh Raj Pant
Senior Veterinary Officer, CVL

Dr. Vinay Kumar Karna
Veterinary officer, CVL

Published by

CENTRAL VETERINARY LABORATORY

Veterinary Complex, Tripureshwor, Kathmandu, Nepal.
Tel : 4261938, 4212143, 4261165
Fax : 4261867
E-mail : cvl@wlink.com.np


Location of CVL, NAL, RVLs and Basic Laboratories (Nepal)
N
CHINA (Tibet)
Central Veterinary Laboratory, Kathmandu
INDIA (Uttar Pradesh)
INDIA (Bihar)
INDIA (Sikkim)
Humla
Mugu
Bajhang
Bajura
Jumla
Dolpa
Mustang
Doti
Achham
Kalikot
Dailekh
Jajarkot
Rukum
Manang
Kailali
Surkhet
Bardia
Salyan
Rolpa
Baglung
Myagdi
Kaski
Gorkha
Lamjung
Banke
Dang
Pyuthan
Gulmi
Syangja
Tanahu
Arghakhanchi
Palpa
Nawalparasi
Chitwan
Makwanpur
Kapilbastu
Rupandehi
Parsa
Bara
Rautahat
Sarlahi
Mahottari
Sindhuli
Ramechhap
Dolakha
Sindhupalchok
Kavrepalanchok
Nuwakot
Rasuwa
Dhading
Patan
Palanchok
Solukhumbu
Bankhuwasabha
Taplejung
Sunsari
Morang
Jhapa
Saptari
Siraha
Udayapur
Dhanusa
Khotang
Bhojpur
Terhathum
Ilam
Panchthar
Central Veterinary laboratory
National Avian Laboratory
Regional Veterinary Laboratories
Basic laboratories

Preface

On behalf of editorial board, I am pleased to present the publication of our Annual Technical Report, 2062/063 in front of you. This issue includes various activities and remarkable works conducted at Central Veterinary Laboratory (CVL), five Regional Veterinary Laboratories (RVLs) and National Avian Laboratory (NAL), Bharatpur during the fiscal year 2062/063 (2005/2006).

We did several remarkable works during last year such as surveillance on Avian Influenza, extension of ELISA test facility in each RVL and NAL, development of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test, setting tissue culture laboratory unit at CVL, and collaboration with international reference laboratories.

Our effort will continue to develop these diagnostic laboratories as centre of excellence. We are in the process of upgrading CVL, NAL and RVLs into Biosafety Level - II laboratory so that CVL would receive accreditation for international certification under ISO.

I would like to express my cordial thanks to all the RVLs as well as NAL for providing their annual progress report and technical articles in due time. I would also like to thank Dr. Poornima Manandhar, Dr. Kedar Bahadur Karki, Dr. Karuna Sharma Bhattarai, Dr. Salina Manandhar and Dr. Pragya Koirala Sharma for their support in publishing this report. My special thanks go to all the technicians of CVL for their sincere contribution and help for providing data based technical information.

Any suggestions for the improvement of its future issue will be highly appreciated.

Dr. Rebati Man Shrestha
Chief Veterinary Officer
Central Veterinary Laboratory

Table of Contents

Central Veterinary Laboratory

Tripureshwor, Kathmandu

1. Introduction

Central Veterinary Laboratory (CVL) works with the objective of securing healthy national herds/flocks of animals and birds throughout the nation based on scientific evidence of the occurrence of diseases of livestock and poultry. Besides, CVL also works on epidemic investigation as well as surveillance and investigation on various diseases/conditions as its approved annual programme. The direct benefit of the performance of various laboratories has been experienced in the field of veterinary medical care based on valid laboratory test results. To achieve these multidimensional activities, CVL works with the application of a series of laboratory test procedures through its various laboratory units; Pathology, Parasitology, Microbiology, Serology, Haematology and Biochemistry units, and Molecular Diagnosis with a considerable progress in the later. At present the molecular based diagnosis of Avian Influenza is in the course of advancement. Similarly, setting up of tissue culture laboratory unit is in progress and expected to conduct virus isolation, identification and sero-typing in shortcoming days.

Central Veterinary Laboratory is always aware in adopting modern disease diagnostic technologies. Endeavour is continuously made in improving its performance in the form of research-oriented activities rather than routine diagnostic works. We are in the process of development of Standard Operating Procedure, test protocols, measurement traceability and biosafety system so that good laboratory practice is followed in our all the diagnostic laboratories. We are already adopting test verification system through international reference laboratories which will help, at least, in the accreditation of CVL for international certification under ISO.

To provide diagnostic facilities throughout the country, CVL works through its five Regional Veterinary Laboratories (RVLs) located one in each of the development regions of the nation; eastern (Biratnagar), central (Janakpur), western (Pokhara), mid-western (Surkhet) and far-western (Dhangadhi) as well as through National Avian Laboratory located in Bharatpur, Chitwan. To provide the diagnostic services smoothly throughout the nation, fifteen basic laboratories established in 15 district livestock service offices (DLSOs) namely, Illam, Jhapa, Saptari, Sarlahi, Rautahat, Parsa, Makawanpur, Kabhre Palanchok, Chitwan, Rupandehi, Dang, Banke, Jumla, Dadeldhura and Kanchanpur, and 60 primary laboratories available one in rest of the DLSOs work in their domain of activities. The basic laboratories are capable to perform microbial culture and antibiotic sensitivity test. Specimens that could not be processed in the aforementioned laboratories due to insufficient facilities are referred to central veterinary laboratory. In this way, CVL works as reference veterinary laboratory in Nepal.

4. Annual programme and progress, CVL (2062/063)

S. N.	Activities	Unit	Target	Budget allocated	Progress	Progress (%)
1	**Laboratory Services**					
1.1	Parasitology	Number	1600	80,000.00	2265	100
1.2	Microbiology	Number	2000	300,000.00	3732	100
1.3	Pathology	Number	1200	255,000.00	1517	100
1.4	Serology	Number	4500	805,000.00	8957	100
1.5	Haematology	Number	500	95,000.00	587	100
1.6	Biochemistry	Number	500	105,000.00	606	100
1.7	Molecular diagnosis	Number	18	230,000.00	26	100
1.8	Rabies diagnosis	Number	30	75000.00	32	100
1.9	Dispatch of samples to other laboratories	Number	500	50,000.00	708	100
2	**Tissue culture laboratory set up**	**Times**	**1**	**200,000.00**	**1**	**100**
3	**Disease investigation and surveillance**					
3.1	Epidemic investigation	Times	12	255,000.00	16	100
3.2	Investigation of Japanese Encephalitis	Times	6	165,000.00	8	100
3.3	Investigation of Khari disease	Times	6	335,000.00	8	100
3.4	Bird flue surveillance	Times	6	75,000.00	19	100
3.5	Sero-surveillance of RP	Times	6	65,000.00	7	100
3.6	PPR surveillance	Times	6	60,000.00	11	100
4	**Serum Bank Management**	**Times**	**12**	**155,000.00**	**12**	**100**
5.	**Teaching Lab Program**					
5.1	Teaching laboratory Management	Times	12	195,000.00	12	100
5.2	Training on laboratory techniques (3 months)	Times	1	300,000.00	1	100
5.3	Training on laboratory techniques (Officer Level 2 weeks in collaboration with CLDP)	Times	1	Budget not received		
6.	**Supervision and Monitoring Program**	**Times**				
6.1	Follow-up & reporting of laboratories	Times	12	85,000.00	13	100
7	**Workshop Program**					
7.1	Workshop on disease investigation in Nepal	Times	1	80000.00	1	100

7.2	Participation in regional workshops (5 regions)	Times	5	40,000.00	6	100
7.3	Workshop on programme & budget of the next F/Y	Times	1	45,000.00	1	100
8	**Publication**					
8.1	Annual technical report (including RVLs & NAL)	Times	1	90000.00	1	100
9	**Contract Service**					
9.1	Sweeper & gardener	Times	3	24000.00	10	100
10	**Purchase**					
10.1	Technical books & journals	Times	3	50,000.00	3	100
10.2	Water bath	Set	1	25,000.00	1	
10.3	Weighing machine	Set	1	50,000.00	1	
10.4	pH meter	Set	1	60,000.00	1	
10.5	Construction of biological pit	Set	1	50,000.00	0	
10.6	Refrigerator	Set	1	75000.00	0	
	Total			**43, 99,000.00**		
	Administrative Expense			**35, 12,000.00**		
	Grand Total			**79, 11,000.00**		
	Total progress in percent					**98**
	Progress weightage in percent					**95.81**

5. Human resource situation, CVL (2062/063)

S. N.	Type of the Post	Class	Number	Fulfilled	Vacant
A.	**Technician (Officer)**				
1.	Chief Veterinary Officer	I	1	1	-
2.	Senior Veterinary Officer	II	2	2	-
3.	Veterinary Officer	III	5	5	-
B.	**Technical (Non-officer)**				
4.	Senior technician	I	7	7	-
5.	Stock man	III	7	7	-
6.	Lab boy	IV	2	-	2
	Total Technical staff		**24**	**22**	**2**
B.	**Non-technician (Non-officer)**				
1.	Junior clerk (Typist)	III	1	1	-
2.	Accountant	I	1	1	-
3.	Clerk	II	1	1	-
4.	Driver	Unclassified	1	1	-
5.	Peon	Unclassified	6	6	-
	Total Administration		**10**	**10**	**-**
	Grand Total		**34**	**32**	**2**

6. Description of human recourse, CVL (2062/063)

S. N.	Name of Staff	Post	Class	Responsibility	Contact
1	**Technical (Officer)**				
1.1	Dr. Rebati Man Shrestha	CVO	I	Chief	9841-209372 Rebati123@yahoo.com
1.2	Dr. Ganesh Raj Pant	SVO	II	Immuno-Serology	4261938 pantganesh@hotmail.com
1.3	Dr. Poornima Manandhar	SVO	II	Microbiology	9841-244884 punicha@yahoo.com
1.4	Dr. Kedar Bahadur Karki	VO	III	Parasitology	9841-258735 karkiked@yahoo.co.in
1.5	Dr. Karuna Sharma	VO	III	Biochemistry	5532394
1.6	Dr. Salina Manandhar	VO	III	Molecular Biology	9841-343927 smanandhar76@yahoo.com
1.7	Dr. Vinay Kumar Karna	VO	III	Pathology	2122246 karnavinay@yahoo.com
1.8	Dr. Pragya Koirala	VO	III	Microbiology	98510-99153 pragya2000@yahoo.com
2.	**Technical (Non-officer)**				
2.1	Mr. Asal Bahadur Tamang	SLT	I	Store	98510-92679 tamangasal@hotmail.com
2.2	Mr. Ashok Prasad Shrestha	SLT	I	Immuno-Serology	98510-98223 ashokptcplus@yahoo.com
2.3	Mr. Gyan Bdr. Bogati	SLT	I	Pathology	5570595
2.4	Mr. Prakash Devkota	SLT	I	Biochemistry	9841-341427 devkota2000@yahoo.co.in
2.5	Mr. Bal Bdr. Kunwar	SLT	I	Microbiology	4258490
2.6	Mr. Tek Bdr. Air	SLT	I	Microbiology	tekair@yahoo.com
2.7	Mr. Shiddha Raj Jaisi	SLT	I	Parasitology	-
3.	**Other technicians**				
3.1	Mr. Purna Maharjan	LT	III	Pathology	4361331
3.2	Mr. Hari Prasad Pyakurel	LT	III	-	4220766
3.3	Mr. Pralhad Basnet	LT	III	Washing & Sterilization	4710772
3.4	Mr. Bhimsen Adhikari	LT	III	Microbiology	9851005008
3.5	Mr. Hari Bhakta Karki	LT	III	Washing & Sterilization	4310186
3.6	Mr. Laxman Sijapati	LT	III	Immuno-Serology	6630677
3.7	Mr. Mukunda Acharya	LT	III	Parasitology	-
4	**Non-technical**				
4.1	Mrs. Kamala Shrestha	Clerk typist	I	Administration	4783533
4.2	Mr. Nim Bdr. Woli	Accountant	I	Account	4811378
4.3	Mr. Laxman Kr. Khanal	Clerk	II	Administration	6206059

4.4	Mr. Macha Kaji Maharjan	Driver	-	Vehicle	4289597
4.5	Mrs. Chiri Maya Maharjan	Helper	-	General Sanitation	-
4.6	Mr. Santa Raj Budhathoki	Helper	-	Parasitology	4286878
4.7	Mrs. Bhima Acharya	Helper	-	Serology	9841-570845
4.8	Mr. Hari Gobinda Shrestha	Helper	-	Pathology	
4.9	Mr. Chandra Bdr. Rana	Helper	-	Rabies laboratory unit	9841-436024
4.10	Mr. Anoj Bajracharya	Helper	-		9841-224085
Technical staff : 22, Non-technical staff : 10, Total : 32					

7. Details of budget sanction and expenditure, CVL (2062/063)

Budget line	Budget head	Budget (Rs.)	
		Sanctioned	Expenditure
1.01	Salary	32,57,000.00	3254504.20
1.02	Allowance	0.0	0.0
1.03	Transfer allowance expense	0.0	0.0
1.04	Clothes	35,000.00	33,000.00
1.05	Food	7,000.00	0.0
2.01	Water and electricity charge	450,000.00	449584.21
2.02	Telecommunication charge	133,000.00	129,759.77
2.03	Official	24,50,000.00	2253079.16
2.05	Repair & maintenance	300,000.00	297,538.35
2.06	Fuel and other provision	260,000.00	259,931.38
2.07	Consultancy and other service charge	19,000.00	18,982.00
2.08	Miscellaneous	60,000.00	59,950.00
4.02	Medicine	135,000.00	128,829.85
4.04	Programme	500,000.00	497,965.00
4.05	Travel & daily allowance (programme)	269,000.00	262,384.00
	Total	78,75,000.00	76,51,787.92
	Salary		70245.00
	Medicine & treatment		142,758.00
	Grand total	**78,75,000.00**	**78,64,790.92**

Pathology Unit

1. Introduction

Pathological test procedure in a biomedical diagnostic laboratory acts as the opening door wherefrom process of disease diagnosis begins. Pathology laboratory unit of central veterinary laboratory includes post mortem unit (necropsy examination) and histopathology unit (histological examination). As a referral veterinary laboratory, CVL receives a large number of specimens from all over the country either directly or through the respective regional veterinary laboratories (RVLs). Besides, district livestock service offices (DLSOs), veterinary practitioners and hatcheries as well as farmers deliver specimens for the purpose of disease diagnosis.

Disease diagnosis starts with necropsy examination in case of dead animals. Besides, history, clinical findings, epidemiological information, and sometimes line of treatment also provide various clues to formulate a tentative diagnosis. Similarly histological examination lays provisional diagnosis of a disease/condition. Therefore, it becomes essential to confirm the case with the application of specific test methods. For this purpose, necropsy technique plays very important role in the procurement of different specimens suitable for various diagnostic techniques. In this way, post mortem unit provides sizeable number of samples to central veterinary laboratory.

Histology unit processes the tissue samples and provides the result over 7-10 days period. Nowadays, this technique is regarded as an obsolete test procedure; nevertheless, its importance has been enormous in a laboratory with limited diagnostic facilities. Its value as a diagnostic procedure is still high in the diagnosis of diseases of neoplastic origin, chronic courses, some of the viral diseases and disease of prion origin where blotting technique is not available. Also its diagnostic value can be looked positively in the use of research works on various diseases.

2. Post-mortem Examination

The total cases received for necropsy examination during F/Y 2062/063 were seven hundred and twenty. Of them, cases of commercial poultry were 664 (92.22%) followed by domestic birds-35 (5.27%), domestic animals-18 (2.71%) and the rest proportion included the cases of common birds (one each of common mynah and crow) and one case of wild bird. Commercial poultry includes cases of broilers-594 (89.45%), layers-51 (7.08%) and parents-19 (2.63%) among the total cases and 91.96%, 7.68% and 2.86% respectively among the total cases of commercial poultry. The present trend of flow of cases of commercial poultry for disease investigation in CVL may indicate the following facts.

Occurrence of various diseases amongst parent stock and layer birds are much less in Kathmandu valley due to scheduled vaccination adopted at hatcheries and layer farms.

Hygienic measures and general management conditions practiced at these farms are improving day-to-day.

Entrepreneurs involved in hatcheries and layer farming might have employed veterinarians and/or technicians at their farms for continuous technical assistance.

Details about the various diseases/conditions tentatively diagnosed in different spp of birds and animals during 2062-063 have been presented in table 1. Similarly, trend in the occurrence of various diseases/conditions tentatively diagnosed in commercial poultry in Kathmandu valley has been presented in table 2.

Table 1 : Diseases/conditions tentatively diagnosed in different spp of birds and animals (F/Y 2062-063)

S. N.	Diseases/Conditions	Species of animals												Total
		Commercial Poultry			Domestic Birds			Common Birds		Wild Bird	Domestic Animals			
		B	L	P	D	Pi	He	Cr	Myn		Pig	Sheep	Goat	
1	Colibacillosis + stress	253	3	1	3	1	1		1	0	0	0	0	263
1.1	Omphalitis	28	0	0	2	0	0	0	0	0	0	0	0	30
1.2	Colisepticaemia	22	0	0	0	0	0	0	0	0	0	0	0	12
1.3	Early chick mortality	9	1	0	0	0	0	0	0	0	0	0	0	10
1.4	Coligranulloma	1	0	0	0	0	0	0	0	0	0	0	0	1
1.6	Colibacillosis + Necrotic enteritis	1	0	0	0	0	0	0	0	0	0	0	0	1
	Sub-total (*E. coli* infestation)	**314**	**4**	**1**	**5**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**327**
2	Mycoses	19	3	0	1	0	0	0	0	0	0	0	0	23
2.1	Mycoses + Colibacillosis	26	0	0	0	1	0	0	0	0	0	0	0	27
2.2	Mycoses + Salmonellosis	1	0	0	0	0	0	0	0	0	0	0	0	1
	Sub-total (Mycoses)	**46**	**3**	**0**	**1**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**52**
3	Infectious bursal disease (IBD)	23	1	0	0	1	0	0	0	0	0	0	0	25
3.1	IBD + Colibacillosis	10	3	0	0	0	0	0	0	0	0	0	0	13
3.2	IBD + ND	1	2	0	0	0	0	0	0	0	0	0	0	3
3.3	IBD + LHD	1	0	0	0	0	0	0	0	0	0	0	0	2
3.4	IBD + Caecal coccidiosis	2	0	0	0	0	0	0	0	0	0	0	0	1
3.5	IBD + CCRD	2	0	0	0	0	0	0	0	0	0	0	0	1
3.6	IBD + Neoplasia	1	0	0	0	0	0	0	0	0	0	0	0	1
	Sub-total (IBD)	**40**	**6**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**47**
4	Salmonellosis	37	4	3	2	0	0	0	0	0	0	0	0	46
5	Chronic respiratory disease (CRD)	8	0	0	0	0	0	0	0	0	0	0	0	8
5.1	Complicated CRD (CCRD)	32	0	2	0	0	0	0	0	0	0	0	0	34
5.2	CCRD + Caecal coccidiosis	1	0	0	0	0	0	0	0	0	0	0	0	1
5.3	CCRD + IB + Mycoses	0	0	1	0	0	0	0	0	0	0	0	0	1
	Sub-total (CRD)	**41**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**44**
6	New Castle disease (ND)	10	3	2	0	2	2	0	0	1	0	0	0	20
6.1	ND + Ascarids	0	1	0	0	1	0	0	0	0	0	0	0	2

6.2	ND + Caecal coccidiosis	1	0	0	0	0	0	0	0	0	0	0	0	1
6.3	ND+ Colisepticaemia	3	0	0	0	0	0	0	0	0	0	0	0	3
	Sub-total (ND)	14	4	2	0	3	2	0	0	1	0	0	0	26
7	Coccidiosis													
7.1	Caecal coccidiosis	16	3	0	0	0	0	0	0	0	0	0	0	19
7.2	Intestinal coccidiosis	2	0	0	0	0	0	0	0	0	0	0	0	2
	Sub-total (Coccidiosis)	**18**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**
8	Bacterial infection	15	2	0	0	0	0	0	0	0	2	0	0	19
9	Fatty Liver Syndrome	18	0	0	0	0	0	0	0	0	0	0	0	18
10	Litchi heart disease (LHD)	13	1	0	1	0	0	0	0	0	0	0	0	15
10.1	LHD + Colibacillosis	1	0	0	0	0	0	0	0	0	0	0	0	1
10.2	LHD + Salmonellosis	0	0	1	0	0	0	0	0	0	0	0	0	1
10.3	LHD + ND + Bacterial Infection	1	0	0	0	0	0	0	0	0	0	0	0	1
	Sub-total (LHD)	**17**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**
11	Pasteurellosis	0	5	1	5	0	0	0	0	0	5	0	0	16
12	Undiagnosed	6	5	0	2	0	1	0	0	0	1	1	0	16
13	Marek's disease (MD)	1	8	2	0	0	1	0	0	0	0	0	0	12
13.1	MD+ Colibacillosis	2	0	0	0	0	0	0	0	0	0	0	0	2
13.2	MD + Ascarids	0	0	1	0	0	0	0	0	0	0	0	0	1
	Sub-total (MD)	**3**	**8**	**3**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**15**
14	Ascarids	0	4	1	0	0	3	0	0	0	0	0	0	8
15	Avian leukosis complex (ALC)	3	1	2	0	0	0	0	0	0	0	0	0	6
15.1	ALC + ND	0	1	0	0	0	0	0	0	0	0	0	0	1
	Sub-total (ALC)	**3**	**2**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**
16	Visceral gout	5	0	1	0	0	0	0	0	0	0	0	0	6
17	Classical swine fever	0	0	0	0	0	0	0	0	0	3	0	0	3
18	Enteritis	2	0	0	1	0	0	0	0	0	0	0	0	3
19	Mineral deficiency	2	0	0	0	0	0	0	0	0	0	0	0	2
20	Mycoplasma synoviae	2	0	0	0	0	0	0	0	0	0	0	0	2
21	Necrotic enteritis	0	1	1	0	0	0	0	0	0	0	0	0	2
22	Sudden death	2	0	0	0	0	0	0	0	0	0	0	0	2
23	Toxicoses	0	0	0	0	0	0	0	0	0	2	0	0	2
24	Transmissible Gastroenteritis	0	0	0	0	0	0	0	0	0	2	0	0	2
25	Aflatoxicoses	1	0	0	0	0	0	0	0	0	0	0	0	1
26	Anthrax	0	0	0	0	0	0	0	0	0	1	0	0	1
27	Avian Encephalomyelitis	1	0	0	0	0	0	0	0	0	0	0	0	1
28	Brooder pneumonia + Omphalitis	1	0	0	0	0	0	0	0	0	0	0	0	1
29	Chicken anaemia agent	1	0	0	0	0	0	0	0	0	0	0	0	1

30	Drug toxicity	1	0	0	0	0	0	0	0	0	0	0	0	1
31	Neoplasia	2	0	0	0	0	0	0	0	0	0	0	0	2
32	Parasitic Gastroenteritis	0	0	0	0	0	0	1	0	0	0	0	0	1
33	Systemic cestodiasis	0	0	0	0	0	0	0	0	0	0	0	1	1
34	Traumatic injury	0	0	0	0	1	0	0	0	0	0	0	0	1
35	Turkey disease	1	0	0	0	0	0	0	0	0	0	0	0	1
36	Vitamin deficiency	1	0	0	0	0	0	0	0	0	0	0	0	1
	Total cases by individual	**594**	**51**	**19**	**18**	**8**	**9**	**1**	**1**	**1**	**16**	**1**	**1**	**720**
	Total cases by type	**664**			**35**			**2**		**1**	**18**			

Table 2 : Trend of disease occurrence in commercial birds in Kathmandu valley

S. N.	Diseases/ Conditions	Months (as per Nepalese fiscal year)												Sub-total			Total
		4	5	6	7	8	9	10	11	12	1	2	3	B	L	P	
1	Colibacillosis + stress	19	2L 34	25	21	22	24	16	20B1 L1P	5	0	20	47	253	3	1	257
1.1	Omphalitis (YSI)	3	3	1	3	0	9	2	1	1	1	4	0	28	0	0	28
1.2	Colisepticaemia	2	2	0	3	1	4	0	3	4	0	2	1	22	0	0	22
1.3	Early chick mortality	0	1	0	3	0	2	1	1	1	1L	0	0	9	1	0	10
1.4	Coligranulloma	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1
1.5	Colibacillosis + NE	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	Sub-total (E. coli)	**25**	**2L 40B**	**26**	**30**	**23**	**40**	**19**	**25B 1L1P**	**11**	**1B 1L**	**26**	**48**	**314**	**4**	**1**	**319**
2	Mycoses	3	3B2 L	0	0	1	0	1L	3	0	0	4	5	19	3	0	22
2.1	Mycoses+ Colibacillosis	7	3	6	3	2		1	0	0	0	2	2	26	0	0	26
2.2	Mycoses+ Salmonellosis	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	Sub-total (Mycoses)	**11**	**6B 2L**	**6**	**3**	**3**	**0**	**1B 1L**	**3**	**0**	**0**	**6**	**7**	**46**	**3**	**0**	**49**
3	Inf. bursal disease	2	2	1L	2	1	1	1	2	3	2	5	2	23	1	0	24
3.1	IBD + Colibacillosis	3L	3	0	0	2	3	1	1	0	0	0	0	10	3	0	13
3.2	IBD + ND	0	0	0	0	0	1	0	0	0	0	1L	1L	1	2	0	3
3.3	IBD + LHD	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1
3.4	IBD + CC	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0	2
3.5	IBD + CCRD	0	1	0	0	0	0	0	1	0	0	0	0	2	0	0	2
3.6	IBD + Neoplasia	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1
	Sub-total (IBD)	**3L 3B**	**8**	**1L**	**2**	**3**	**5**	**2**	**4**	**4**	**2**	**5B 1L**	**2B 1L**	**40**	**6**	**0**	**46**
4	CRD	1	2	0	3	0	0	0	1	1	0	0	0	8	0	0	8
4.1	Complicated CRD	5	1	1	0	6	5	5	3B 2P	1	0	5	0	32	0	2	34
4.2	CCRD + CC	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1

4.3	CCRD + IB + Mycoses	1P	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	Sub-total (CRD)	**1P 6B**	**3**	**1**	**3**	**7**	**5**	**5**	**4B 2P**	**2**	**0**	**5**	**0**	**41**	**0**	**3**	**44**
5	Salmonellosis	7	4B 1L	1L1 BP2	2	2B 1P	6	3	3	2B 1L	1L1 BP1	2	3	37	4	3	44
6	Coccidiosis																
6.1	Caecal coccidiosis (CC)	1L	2	1B 1L	1	5B 1L	0	2	1	1	2	0	1	16	3	0	19
6.2	Intestinal coccidiosis (IC)	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0	2
	Sub-total (Coccidiosis)	**1L 1B**	**3**	**1B 1L**	**1**	**5B 1L**	**0**	**2**	**1**	**1**	**2**	**0**	**1**	**18**	**3**	**0**	**21**
7	New Castle disease	1L 1B	1L	0	1B 1L	1P	2	1	1	3	0	1B1 P	0	10	3	2	15
7.1	ND + Ascariasis	0	1 L	0	0	0	0	0	0	0	0	0	0	0	1	0	1
7.2	ND + CC	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
7.3	ND + Colisepticaemia	0	0	0	0	1	2	0	0	0	0	0	0	3	0	0	3
	Sub-total (ND)	**2B 1L**	**2L**	**0**	**1B 1L**	**1B 1P**	**4**	**1**	**1**	**3**	**0**	**1B 1P**	**0**	**14**	**4**	**2**	**20**
8	Fatty Liver Syndrome	1	1	0	4	1	4	6	1	0	0	0	0	18	0	0	18
8.1	Litchi heart disease	1	1	1	2	0	0	0	0	2	0	2	4	13	0	0	13
8.2	LHD + Colibacillosis	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1
8.3	LHD + Salmonellosis	0	0	0	0	0	0	0	0	0	1P	0	0	0	0	1	1
8.4	LHD + ND + BI	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	2
8.5	LHD + CRD	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	Sub-total (LHD)	**1**	**2**	**1**	**2**	**0**	**2**	**0**	**0**	**3**	**1P**	**2**	**4**	**17**	**0**	**1**	**18**
9	Bacterial infection (BI)	2	2B 1L	1	1L	2	3	1	2	1	0	0	1	15	2	0	17
9.1	Marek's disease (MD)	1L 1P	3L	1L	1P	0	1L	0	1B 1L	0	1L	0	0	1	8	2	11
9.2	MD+ Colibacillosis	0	0	0	0	2B	0	0	0	0	0	0	0	2	0	0	2
9.3	MD + Ascariasis	1P	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	Sub-total (MD)	**1L 2P**	**3L**	**1L**	**1P**	**2B**	**1L**	**0**	**1B 1L**	**0**	**1L**	**0**	**0**	**3**	**8**	**3**	**14**
10	Undiagnosed	2L 1B	0	0	0	0	1L	1B 1L	0	0	1	1	1B 2L	6	5	0	11
11	Avian leukosis complex	1L	0	0	1	1B 1P	0	0	0	0	0	1P	1	3	1	2	6
11.1	ALC + ND	1L	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

	Sub-total (ALC)	2L	0	0	1	1B 1P	0	0	0	0	0	1P	1	3	2	2	7
12	Pasteurellosis	1L	1P	0	0	0	1L	0	1L	1L	1L	0	0	0	5	1	6
13	Visceral gout	0	1	1	2	1	0	0	1P	0	0	0	0	5	0	1	6
14	Ascariasis	0	1P	0	0	1L	0	0	0	0	0	1L	2L	0	4	1	5
15	Enteritis	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0	2
16	Mineral deficieny	0	0	0	0	0	0	1	0	1	0	0	0	2	0	0	2
17	M. synoviae infecion	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0	2
18	Necrotic enteritis (NE)	0	0	0	0	0	0	0	0	0	1L	1P	0	0	1	1	2
19	Neoplasia	1	0	0	1	0	0	0	0	0	0	0	0	2	0	0	2
20	Sudden death	1	0	0	1	0	0	0	0	0	0	0	0	2	0	0	2
21	Aflatoxicoses	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1
22	AE	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1
23	Aspergillosis + YSI	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
24	C. anaemia agent	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
25	Drug toxicity	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1
26	Turkey disease	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
27	Vitamin deficiency	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1
	Total	**76**	**85**	**47**	**58**	**57**	**73**	**44**	**52**	**30**	**14**	**53**	**75**	**594**	**51**	**19**	**664**

Note : 1. B- broiler, L- layer, P- parent, D- duck, Pi- pigeon, He- hen, Cr- crow, Myn- mynah (Table 1).

2. L stands for layers and P for parents. The number without L or P should be understood for the corresponding number of broilers (Table 2).

3. Histological Examination

Histopathology laboratory unit received a total of seventy-two sets of specimen from different species of animals and birds during 2062/063. These specimens comprised 35 (48.61%) cases of commercial poultry, 16 (22.22%) cases of dogs, 10 (13.88%) cases of pigs, three cases (4.16%) of goats, two cases (2.77%) each for buffalo, sheep and duck, and one case (1.38%) each for turkey and pigeon.

Among the total cases of commercial poultry, twenty-three cases were received from broilers, eight cases from layers and five cases from parent stocks. The four cases of parent stocks were presumably diagnosed for visceral form of Marek's disease and one case for lymphoid leukosis. Similarly, the two cases of layers were diagnosed for classical form of Marek's disease, four cases for visceral form of Marek's disease, one case for fowl pox and rest one as the case of generalized inflammation. In case of broilers, one each case of visceral form of Marek's and lymphoid leukosis was diagnosed while five cases of tumorous growth were diagnosed as neoplasia of unknown etiology. Similarly, six cases were diagnosed as hydropericardium hepatitis syndrome, six cases as acute inflammation, one case of avian encephalomyelitis, two cases of inclusion body hepatitis and one of the cases undiagnosed.

Similarly, among the sixteen cases of canine, two cases of papillomma and one case was diagnosed each for meningiomma and chronic inflammation and rest 12 cases were diagnosed for generalized nephritis, interstitial nephritis and glomerulonephritis.

Of the 10 cases of swine, one case could be only interpreted for the occurrence of Japanese encephalitis with ochratoxicosis. Rest of the specimens was diagnosed simply as the cases of acute and mild inflammations. The histological interpretation of 3 cases of goats could not be made due to irrelevant specimens and choice of test methodology.

The two cases of buffalo were diagnosed one each as case of poisoning and cirrhosis of liver. Similarly, one case of duck was diagnosed as hepatitis while one case could not be diagnosed. The cases of sheep were diagnosed as emphysema of lungs and chronic inflammatory response. The one case each of pigeon was diagnosed as avian encephalomyelitis and the case of turkey could not be interpreted.

It may be mentioned that the histopathological study of tissues/organs has ever been realized difficult. In most of the cases, various specimens are interpreted simply as general pathological conditions for which important diseases of animals and birds remain undiagnosed. Following are some of the important justifications why histopathological studies have been difficult.

Inadequate Specimen Information

Most of the specimens received are provided with inadequate information on pathogenesis, clinical features and epidemiological information of the diseases, and post mortem examination of an individual dead animal or bird.

Irrelevant or Inadequate Specimen/Sample

It has also been experienced as a great problem. In most of the cases, either the available specimen does not match with the test prescribed by clients or the specimen lacks the support tissues/organs necessary for studying the appropriate lesions for a disease in question.

Improper Test Method Selection

Selection of test methods also needs to be improved. In most instances, cases diagnosed as Colibacillosis are referred to histology unit rather than bacteriological culture and identification.

Serology Unit

1. Introduction

Serology unit is responsible to conduct various serological tests to detect antigen and antibody for the purpose of diagnosis, screening, monitoring and surveillance of bacterial and viral diseases of animal and poultry. Similarly this unit is performing competitive enzyme-linked immunosorbent assay (C-ELISA) test to measure the antibody titer in response to Goat plague vaccine in sheep and goat under National Peste des petits ruminant (PPR) control Program. We have introduced C-ELISA and IgM Capture ELISA test for sero-survey of Avian Influenza A virus and Japanese encephalitis virus receptively. Other serological test used in this laboratory are agar gel immunodiffusion (AGID), plate agglutination test (PAT), solid phase immuno assay and rapid antigen detection test. Serum samples are received from RVLs, NAL, DLSOs, commercial poultry farms, private veterinary practitioners, farmers and pathology unit of our own laboratory. Samples thus received are tested in serology laboratory unit and more often, the test results are reconfirmed from OIE reference laboratories as per need.

2. Programmes and Progress

The result of different test for specific disease has been presented from table 1-10 with the overall progress in table 11. A total number of 6228 sera were tested during 2062/063 and 32.91% of the samples were found test positive. Of the total samples, 2484 sera intended for seromonitoring were tested to detect antibodies in response to PPR live vaccine or PPR infection of which 39.33% were found positive. However, the percentage of positive was very low which suggests the demand of effective PPR vaccination programme to control the disease in the country (Table 1). These sera were collected from 47 districts and samples from 42 districts were found positive whereas samples from five districts remained negative.

Screening for Brucellosis was conducted in six districts by testing 81 samples collected from cattle, buffaloes, pigs and dogs. Only three samples one each for buffalo, cattle and dog received from Dhading, Rupendehi and Kathmandu district were found positive on PAT receptively (Table 2).

Antibodies against salmonella infestation were detected in Chitwan, Bhaktapur, and Nawalparasi and Saptari districts when 372 poultry serum samples were tested by conducting plate agglutination test. Among them, 8.06% samples were found positive threatening the poultry production of country (Table 3). Prevalence of Mycoplasmosis was detected 7.14-34.41 % in PAT and immunocomb test (Table 4, 8 & 9). Antibodies against infectious bursal disease, Newcastle disease and infectious bronchitis were detected 89.96, 96.11 and 90.93 % in vaccinated flock that suggests good vaccination program in commercial poultry (Table 5, 6 and 7).

Under avian influenza A surveillance program, 1518 samples comprising cloacal and pharyngeal swab from domestic and wild birds were tested to detect avian influenza A viral antigen with the use of commercially available rapid antigen detection (SD bioline, Korea, AIV Ag and Synbiotic, USA) test kits. This work was jointly conducted by serology and virology laboratory units of

CVL. None of the tested samples were found positive for avian influenza viral antigen (Table 8). Meanwhile, 96 sera were tested by performing haemagglutination inhibition (HI) test and 114 samples were tested by performing C-ELISA (Table 9 & 10) to detect antibody against the same virus virus (AI). All samples tested with HI were negative for AI where as nine sera tested with C-ELISA was found positive for antibody against AI virus. In this way antibodies against AI in domestic poultry of Nepal were first detected at CVL by performing C-ELISA. The positive samples were sent to OIE reference laboratory for avian influenza for the confirmation and the result were found same as in CVL. Meanwhile reference laboratories also detected antibodies against H9 subtype of AI virus in Nepalese poultry. However, Nepal is free from Bird flu or Highly Pathogenic Avian Influenza as all the tested sera, swabs and tissue samples were found negative against H5 and H7 virus in serology, virus isolation, RT-PCR and immunochemistry test performed at CVL and OIE reference laboratories (Italy, Australia and UK).

The finding of sero-survey of Japanese encephalitis had been much interesting in 2062/063. A total 114 sera were collected from pigs, horses, ducks and human and subjected to C-ELISA test. The prevalence of JE was 71.92 %. We were also able to detect antibodies (IgG) against JE in humans for the first time in CVL as well as in Nepal with C-ELISA. Tested human sera were collected from hospitalized patients who were clinically suffering from JE infection (Table 7). Therefore this test would be useful to monitor the antibodies in vaccinated human population in future and would be a useful tool for human health authority.

Table 1 : PPR, C-ELISA test result (2062/063)

S.N.	Districts	Species	Total Sample	Positive	Negative
1	Lalitpur	Goat	2	0	2
2	Makwanpur	Goat	48	19	29
3	Mahottari	Goat	112	56	56
4	Palpa	Goat	6	6	0
5	Kathmandu	Goat	110	77	33
6	Bhaktapur	Goat	61	38	23
7	Sindhupalchowk	Goat	45	25	20
8	Saptari	Goat	98	42	56
9	Dhanusha	Goat	124	25	99
10	Khotang	Goat	97	0	97
11	Jhapa	Goat	60	22	38
12	Morang	Goat	79	21	58
13	Kailali	Goat	17	2	15
14	Sarhahi	Goat	31	19	12
15	Sunsari	Goat	50	27	23
16	Siraha	Goat	127	64	63
17	Kapilbastu	Goat	141	70	71
18	Bara	Goat	21	6	15
19	Ramechhap	Goat	10	4	6
20	Kanchanpur	Goat	243	60	183

21	Kaski	Goat	20	8	12
22	Ilam	Goat	63	11	52
23	Banke	Goat	263	73	190
24	Dadeldhura	Goat	12	4	8
25	Nawalparashi	Goat	17	7	10
26	Gulmi	Goat	13	7	6
27	Shyanja	Goat	3	0	3
28	Okahadungha	Goat	10	1	9
29	Udayapur	Goat	24	10	14
30	Arghakanchi	Goat	21	7	14
31	Rupendehi	Goat	29	15	14
32	Rashuwa	Goat	68	19	49
33	Parsha	Goat	38	11	27
34	Rukum	Goat	79	51	28
35	Shalyan	Goat	101	57	44
36	Kalikot	Goat	16	9	7
37	Surkhet	Goat	7	0	7
38	Dolakha	Goat	24	8	16
39	Chhitawn	Goat	3	3	0
40	Baglung	Goat	2	2	0
41	Humla	Goat	83	55	28
42	Bhojpur	Goat	31	0	31
43	Dhading	Goat	3	3	0
44	Mugu	Goat	40	18	22
45	Myangdi	Goat	5	2	3
46	Tanahu	Goat	5	3	2
47	Rautahat	Goat	22	10	12
Total			**2484**	**977**	**1507**

Table 2 : PAT test result for Brucellosis (2062/063)

S.N.	Districts	Species	Total sample	Positive	Negative
1	Rupandehi	Cattle	27	1	26
2	Kathmandu	Dog	4	1	3
3	Makwanpur	Pig	22	0	22
4	Dhading	Buffalo	6	1	5
5	Chitawn	Buffalo	2	0	2
6	Gulmi	Cattle	20	0	20
Total			**81**	**3**	**78**

Table No. 3 : PAT test result for Salmonellosis (2062/063)

S.N.	Districts	Species	Total sample	Positive	Negative
1	Chitwan	Poultry	217	20	197
2	Bhaktapur	Poultry	26	8	18
3	Kathmandu	Poultry	22	0	22
4	Nawalparasi	Poultry	38	1	37
5	Mohattari	Poultry	6	0	6
6	Dhanusha	Poultry	6	0	6
7	Sunsari	Poultry	33	0	33
8	Rupandehi	Poultry	9	0	9
9	Saptari	Poultry	15	1	14
Total			**372**	**30**	**342**

Table 4 : PAT test result for Mycoplasmosis (2062/063)

S.N.	District	Species	Tested samples	Positive	Negative
1	Chitwan	Poultry	56	4	52

Table 5 : Immunocomb test result for IBD, ND and IB of Poultry (2062/063)

S.N.	District	Total sample	IBD		ND		IB	
			+ve	-ve	+ve	-ve	+ve	-ve
1	Chitawn	171	158	13	169	2	156	15
2	Bhaktapur	20	16	4	20	0	19	1
3	Kathmandu	33	33	0	33	0	33	0
4	Nawalparasi	39	39	0	39	0	39	0
5	Kaski	10	9	1	10	0	10	0
6	Mohottari	6	3	3	3	3	0	6
7	Dhanusha	6	1	5	2	4	2	4
8	Rupandehi	9	5	4	6	3	7	2
9	Saptari	15	14	1	15	0	15	0
Total		**309**	**278**	**31**	**297**	**12**	**281**	**28**

Table 6 : Immunocomb test result for MG and MS of Poultry (2062/063)

S.N.	District	Total sample	M. gallisepticum		M. synovae	
			+ve	-ve	+ve	-ve
1	Chitawn	118	32	86	15	103
2	Bhaktapur	20	7	13	0	20
3	Kathmandu	7	0	7	0	7
4	Nawalparasi	39	25	14	1	38
5	Kaski	10	1	9	1	9
6	Mohottari	6	0	6	0	6
7	Dhanusha	6	0	6	0	6
8	Rupandehi	9	9	0	1	8
Total		**215**	**74**	**141**	**18**	**197**

Table 7 : C-ELISA test result for Japanese encephalitis virus infection (2062/063)

S.N.	District	Pig		Horse		Duck		Human	
		Total	+ve	Total	+ve	Total	+ve	Total	+ve
1	Banke	8	7	9	9	7	0	4	1
2	Bardiya	0	0	0	0	0	0	5	2
3	Dang	0	0	0	0	0	0	3	0
4	Kailali	27	26	0	0	0	0	19	10
5	Makwanpur	32	27	0	0	0	0	0	0
Total		**67**	**60**	**9**	**9**	**7**	**0**	**31**	**13**

Table 8 : Avain influenza antigen detection test result (2062/063)

Test Date	District	Species	Type of Sample	Total Sample	Test Name	Sample Tested	Results	
							Negative	Positive
062/7/3	Gorkha	Pegion	Cloacal swab	50	RADT	25	25	0
062/7/21	Banke	Chicken	Cloacal swab	16	RADT	8	8	0
062/7/22	Bhaktapur	Chicken	Cloacal swab	19	RADT	11	11	0
062/7/21	Kathmandu	Pegion	Cloacal swab	3	RADT	1	1	0
062/7/21	Kathmandu	Chicken	Cloacal swab	48	RADT	34	34	0
062/8/3	Chitwan	Chicken	Cloacal swab	9	RADT	6	6	0
062/8/3	Mohottari	Pegion	Cloacal swab	12	RADT	8	8	0
062/8/3	Sunsari	Sparrow	Cloacal swab	5	RADT	2	2	0

062/08/08	Chitwan	Chicken	Cloacal swab	55	RADT	40	40	0
062/09/15	Bara	Chicken	Cloacal swab	5	RADT	3	3	0
062/09/16	Parsa	Chicken	Cloacal swab	5	RADT	3	3	0
062/09/15	Makwanpur	Chicken	Cloacal swab	5	RADT	3	3	0
062/10/27	Nawalparasi	Chicken	Tracheal swabs	30	RADT	30	30	0
062/11/15	Nuwakot	Chicken	,,	20	RADT	11	11	0
062/11/22	"	"	Tracheal swab	3	RADT	3	3	0
062/12/08	Morang	Pegion	T.Swab	10	RADT	10	10	0
,,	"	"	Cloacal swab	3	RADT	3	3	0
"	"	"	Tracheal swab	3	RADT	3	3	0
"	"	"	Cloacal swab	1	RADT	1	1	0
"	"	"	Tracheal swab	2	RADT	2	2	0
"	"	"	Cloacal swab	2	RADT	2	2	0
"	"	"	Tracheal swab	2	RADT	2	2	0
062/11/29	Kanchanpur	Malewa	Tracheal swab	1	RADT	1	1	0
062/12/01	Banke	Chicken	Cloacal swab	4	RADT	4	4	0
,,	,,	Duck	Cloacal swab	7	RADT	7	7	0
062/12/03	Kathmandu	Wild bird	Tracheal swab	6	RADT	6	6	0
,,	,,	Wild bird	C.Swab	4	RADT	4	4	0
,,	,,	Duck	T.Swab	3	RADT	3	3	0
062/12/05	,,	Chicken	T.Swab	11	RADT	11	11	0
,,	,,	,,	C.Swab	11	RADT	11	11	0
062/12/06	Morang	Crow	T.Swab	1	RADT	1	1	0
,,	Morang	Wild bird	T.Swab	3	RADT	3	3	0
,,	Kathmandu	Duck	T.Swab	3	RADT	3	3	0
062/11/28	,,	Wild bird	T.Swab	6	RADT	6	6	0
,,	,,	Wild bird	C.Swab	4	RADT	4	4	0
062/12/08	Makwanpur	Poultry	C. Swab	4	RADT	4	4	0
,,	Dhanusha	Duck	C.Swab	6	RADT	6	6	0
,,	Bara	Poultry	C.Swab	5	RADT	5	5	0

„	Parsa	Poultry	C.Swab	5	RADT	5	5	0
„	Lalitpur	Pegion	T.Swab	2	RADT	2	2	0
„	Morang	Pegion	T.Swab	6	RADT	6	6	0
„	Morang	Poultry	T.Swab	4	RADT	4	4	0
„	„	Duck	T.Swab	2	RADT	2	2	0
„	„	Pig	T.Swab	2	RADT	2	2	0
„	„	Crow	T.Swab	1	RADT	1	1	0
062/12/10	Sarlahi	Poultry	T.Swab	7	RADT	7	7	0
„	„	„	C.Swab	7	RADT	7	7	0
„	Morang	Pegion	T.Swab	8	RADT	8	8	0
„	„	Poultry	T.Swab	8	RADT	8	8	0
„	„	Duck	T.Swab	8	RADT	8	8	0
„	„	Crow	T.Swab	2	RADT	2	2	0
„	„	Wild bird	T.Swab	2	RADT	2	2	0
062/12/13	Morang	Poultry	T.Swab	3	RADT	3	3	0
„	„	Duck	T.Swab	6	RADT	6	6	0
„	„	Pegion	T.Swab	2	RADT	2	2	0
„	Kailali	Poultry	T.Swab	4	RADT	4	4	0
062/12/18	Kapilbastu	Poultry	T.Swab	10	RADT	10	10	0
„	„	„	C.Swab	10	RADT	10	10	0
„	„	Crow	C.Swab	1	RADT	1	1	0
063/1/19	Kanchanpur	Poultry	C.Swab	5	RADT	5	5	0
063/1/19	Kanchanpur	Poultry	T.Swab	5	RADT	5	5	0
063/1/19	Kanchanpur	Poultry	C.Swab	5	AIV H5	5	5	0
063/1/19	Kanchanpur	Poultry	T.Swab	5	AIV H5	5	5	0
063/1/19	Kanchanpur	Poultry	C.&T. Swab	5	RADT	5	5	0
063/1/23	Kanchanpur	Poultry	C.&T. Swab	10	RADT	10	10	0
063/1/25	Kanchanpur	Poultry	C.&T. Swab	10	AIV H5	10	10	0
063/1/26	Kanchanpur	Poultry	C.&T. Swab	10	RADT	10	10	0
063/1/27	Kanchanpur	Poultry	C.&T. Swab	10	RADT	10	10	0
063/1/28	Kanchanpur	Poultry	C.&T. Swab	10	AIV H5	10	10	0
063/02/01	Kanchanpur	Poultry	C.&T. Swab	5	RADT	5	5	0
063/02/01	Kanchanpur	Poultry	C.&T. Swab	5	RADT	5	5	0
063/02/01	Kanchanpur	Poultry	C.&T. Swab	5	AIV H5	5	5	0
063/02/25	Kathmandu	Wild birds	Faeces	35	RADT	20	20	0

063/04/03	Dadeldhura	Poultry	Cloacal swab	9	RADT	3	3	0
063/04/03	Dhangadhi	Poultry	Tracheal Swab	51	RADT	9	9	0
063/03/29	Shyangja	Poultry	Cloacal swab	4	RADT	1	1	0
063/03/29	Gorakha	Poultry	Cloacal swab	20	RADT	8	8	0
Total				**5152**		**1518**	**1518**	**0**

Table 9 : Avain influenza antibody detection test result (2062/063)

Test date	District	Species	Sample	Total sample	Name of test	Sample tested	Negative	Positive
062/7/4	Gorakha	Pegion	Serum	35	HI	22	22	0
062/7/13	Kathmandu	Chicken	Serum	25	HI	20	20	0
062/7/13	Gorakha	Pegion	Serum	16	HI	10	10	0
062/7/13	Morang	Chicken	Serum	16	HI	10	10	0
062/7/13	Chitawan	Chicken	Serum	28	HI	20	20	0
062/7/13	Kaski	Chicken	Serum	16	HI	10	10	0
062/7/13	Dhanusha	Chicken	Serum	6	HI	4	4	0
Total				**142**		**96**	**96**	**0**

Table 10 : Avain influenza antibody detection test result (2062/063)

Test date	District	Species	Sample	Total sample	Name of test	Sample tested	+ve	-ve
062/725	Chitawan	Chicken	Serum	15	C-ELISA	15	1	14
062/7/25	Kathmandu	Chicken	Serum	10	C-ELISA	10	0	10
062/7/25	Kaski	Chicken	Serum	8	C-ELISA	8	0	8
062/7/25	Mahottari	Chicken	Serum	6	C-ELISA	6	0	6
062/7/25	Dhanusha	Chicken	Serum	1	C-ELISA	1	0	1
62/11/18	Chitawan	Chicken	Serum	40	C-ELISA	40	4	36
063/1/13	Chitawan	Chicken	Serum	10	C-ELISA	10	0	10
063/1/13	Kailali	Chicken	Serum	16	C-ELISA	16	1	15
063/1/13	Banke	Chicken	Serum	14	C-ELISA	14	1	13
063/3/15	Kanchanpur	Chicken	Serum	19	C-ELISA	19	2	17
063/3/15	Nuwakot	Chicken	Serum	15	C-ELISA	15	0	15
063/3/15	Rasuwa	Chicken	Serum	6	C-ELISA	6	0	6
Total				**160**		**160**	**9**	**151**

Table 11 : Summary of serological test results (2062/063)

S. N.	Disease	Test method	Sample tested	Test results		Positive (%)
				Positive	Negative	
1	Goat plague	C-ELISA	2484	977	1507	39.33
2	Buucellosis	PAT	81	3	78	3.7
3	Salmonellosis	PAT	372	30	342	8.06
4	Mycoplasmosis	PAT	56	4	52	7.14
5	Infectious Bursal Disease	Immunocomb	309	278	31	89.96
6	Newcastle Disease	Immunocomb	309	297	12	96.11
7	Infectious Bronchitis	Immunocomb	309	281	28	90.93
8	Mycoplasmosis (MG)	Immunocomb	215	74	141	34.41
9	Mycoplasmosis (MS)	Immunocomb	215	18	197	8.37
10	Avian Inflenza	RADT	1518	0	1518	0
11	Avian Inflenza	HI	96	0	96	0
12	Avian Inflenza	C-ELISA	160	9	151	5.62
13	Japanese encephalitis	C-ELISA	114	82	32	71.92
	Total		**6238**	**2053**	**4185**	**32.91**

Microbiology Unit

1. Introduction

This unit is responsible for routine diagnosis and investigation of epidemics. In addition, it involves in research and development activities such as antigen production and development of test procedures; penside test for the diagnosis of Peste des Petits Ruminanats. Salmonella antigen thus produced is supplied to different RVLs and private practitioners on demand. Similalry, we are also involved in the thesis works of graduate and post-graduate students received from various academic institutions. Recently the role and responsibilities of this unit has been expanded in the surveillance, isolation and diagnosis of avian influenza, strain identification of Newcastle disease and diagnosis of swine fever.

Microbiology unit receives a wide variety of samples from field condition, veterinary hospitals, farmers, DLSOs, animal quarantine check-posts and post mortem unit of our own laboratory. Besides, we receive primary isolates from RVLs as well as NAL for reconfirmation and result verification. Microbiology laboratory unit comprises four sub-units; bacteriology and mycology, virology, rabies diagnosis unit, and washing and sterilization unit through which various activities are as performed in the unit.

2. Programmes and progress of various sub-units

2.1 Bacteriology and mycology unit

This unit is responsible for isolation and identification of bacteria and fungi from from various diseases/conditions. It also performs drug sensitivity test to the isolated organism that facilitate the proper line of treatment. The major samples include milk, various tissues, eggs (commercial poultry), blood and urine followed by swabs, and pus from different species of animal. Similarly water samples are also received from different hatcheries for the appreciation of microbes present therein. This unit receives necropsies from commercial poultry for fungal isolation from the post mortem laboratory unit of CVL and occasionally from RVLs for reconfirmation and verification.

Progress

During the F/Y 2062/063, a total of 1181 samples were received from different species of animals. Among them, 876 samples were found positive from which different organisms were isolated through different types of culture methods. Out of these positive samples, different species of fungus were isolated from 41 samples.

A total sample received from different species of animals and birds from post mortem unit of CVL was 620 which included 565 samples from poultry, 16 samples from duck, 15 from swine, eight from bovine, five from pigeon, three samples each from caprine and equine, and one each from canine, parrot, rupi (a wild bird) and turkey. Various organisms like *E. coli*, *Salmonella* spp, *Staphylococcus* spp, *Streptococcus* spp and *Pasteurella* spp were isolated from 519 positive samples

as shown in table 1. This table also reveals that more than one bacterium has been isolated from a single sample.

Table 1 : Result of bacteriological culture of post mortem samples (CVL)

S.N.	Species	Total	+ve	-ve	Isolates	*No.
1	Poultry	565	476	89	*E. coli* *Salmonella* spp *Staphylococcus* spp *Streptococcus* spp *Pasteurella* spp Others (*Diplococcus* spp, *Bacillus* spp, *proteus* spp)	307 121 179 43 10 4
2	Duck	16	15	1	*E. coli* *Staphylococcus* spp *Pasteurella* spp Others (*Salmonella* spp, *Proteus* spp)	6 8 4 2
3	Swine	15	11	4	*E.coli* *Pasteurella* spp *Staphylococcus* spp *Streptococcus* spp	7 1 5 3
4	Bovine	8	8	-	*E.coli* *Staphylococcus* spp *Proteus* spp	7 5 1
5	Pigeon	5	3	2	*E.coli* *Staphylococcus* spp	2 3
6	Mare	3	3	-	*Staphylococcus* spp *Bacillus* spp	2 2
7	Caprine/Ovine	4	1	3	*E.coli*	1
8	Canine	1	1	-	*Stophylococcus* spp	1
9	Parrot/Rupi/Turkey	3	1	2	*E.coli*	1
Total		**620**	**519**	**101**		

*(Note : * No. represents the number of isolates in table 2.1.1-2.1.11).*

A total of 380 milk samples received from field condition. Out of them, 266 samples were screened as positive through California Mastitis Test (CMT). The major isolates were ***Staphylococcus, E.coli, Streptococcus*** and ***Klebsiella. Bacillus*** was also isolated from few milk samples. The various isolates of milk samples are presented in table 2.

Table 2 : Result of bacteriological culture of milk samples

Species	Total	Positive	Negative	Isolates	No.
Bovine	380	266	114	*E.coli* *Staphylococcus* spp *Streptococcus* spp *Klebsiella* spp *Bacillus* spp	142 183 38 11 2
	380	**266**	**114**		

Similarly, a total of 10 vaginal swabs of bovine were received from field condition and the various isolates from nine positive samples were *Staphylococcus, Klebseilla. E.coli, Bacillus and Pseudomonas*, the details of the result has been shown in table 3.

Table 3 : Result of bacteriological culture of vaginal swabs

Species	Total	Positive	Negative	Isolates	No.
Bovine	10	9	1	*Staphylococcus* spp *Klebsiella* spp *E.coli* *Bacillus* spp *Pseudomonas* spp	2 5 1 2 1
	10	**9**	**1**		

The total blood samples received from field condition were sixty-five constituting 37 samples of canine and 21 samples of bovine (21), and three each of swine and equine. Most of these samples were found negative with only nine samples positive. The detail of the result has been given in table 4.

Table 4 : Result of bacteriological culture of blood samples

S.N.	Species	Total	Positive	Negative	Isolates	No.
1.	Bovine	21	1	20	*Pasteurella* spp	1
2.	Canine	37	5	32	*E.coli* *Haemophillus* spp	4 1
3.	Swine	3	-	3	-	-
4.	Equine	4	-	4	-	-
Total		**65**	**6**	**59**		

Only two ear swabs from canine were received with all positive for the presence of *Staphylococcus* as shown in table 5.

Table 5 : Result of Bacterial Culture of Ear Swab Samples

Species	Total	Positive	Negative	Isolates	No.
Canine	2	2	-	*Staphylococcus* spp	2

The total number of urine samples of different species received from field condition was thirteen. Among them, 10 samples were found positive with the presence of various isolates like *Staphylococcus* spp, *E.coli* and *Streptococcus* spp. The result of bacteriological culture of urine samples has been shown in table 6.

Table 6 : Result of bacteriological culture of urine

S.N.	Species	Total	Positive	Negative	Isolates	No.
1.	Bovine	8	5	3	*Staphylococcus* spp *Streptococcus* spp *E.coli*	4 1 4
2.	Canine	4	4	-	*Staphylococcus* spp *Streptococcus* spp *E.coli*	2 1 2
3.	Ovine	1	1	-	*Staphylococcus* spp	1
Total		**13**	**10**	**3**	-	

Similalry, the total pus samples and nasal swabs received from field conditions were four and seven respectively. Among the pus, only two samples of canine were positive revealing the presence of *Staphylococcus* spp and are given in table 7. The total nasal swabs found to be positive were five showing the presence of *Staphylococcus* spp and E.*coli* and the result is given in table 8.

Table 7 : Result of Pus Culture

S.N.	Species	Total	Positive	Negative	Isolates	No.
1.	Canine	3	2	1	*Staphylococcus* spp *Streptococcus* spp	2 1
2.	Swine	1	-	1	-	
Total		**4**	**2**	**2**	-	

Table 8 : Cultural results of nasal swab

Species	Total	Positive	Negative	Isolates	No.
Bovine	7	5	2	*Staphylococcus* spp *E.coli*	5 3

The samples of body fluid from bovine were received from field condition were two with all positive and the result is shown in table 9.

Table 9 : Result of bacterial culture of body fluid

Species	Total	Positive	Negative	Isolates	No.
Bovine	2	2	-	*Staphylococcus* spp *E.coli*	2 2

The Lab also received water samples from different hatcheries. During 2062/063, a total of 20 water samples were received with 16 samples found positive and four negative. The various isolates are given in table 10.

Table 10 : Result of bacterial culture of water

Species	Total	Positive	Negative	Isolates	No.
Water	20	16	4	*Staphylococcus* spp *E.coli* *Streptococcus* spp	7 7 2
	20	**16**	**4**	-	**14**

The total sample intended for fungal culture were fifty-eight. Among them, 37 samples were received from poultry and rest from canine, bovine, swine, pigeon and caprine. The result of isolated fungi is shown in given table 11.

Table 11 : Result of Fungal culture of different species of animals

S. N.	Species	Total	Positive	Negative	Isolate Fungus	No.
1.	Avian	37	31	6	*Penicillium* spp *Candida* spp	27 4
2.	Canine	9	4	5	*Penicillium* spp	4
3.	Bovine	5	4	1	*Penicillium* spp *Mucor* spp	3 1
4.	Swine	4	2	2	*Candida* spp *Aspergillus* spp	1 1
5.	Pigeon	2	-	2	-	-
6.	Caprine	1	1	-	*Penicillium* spp	1
Total		**58**	**42**	**16**		

2.2 Virology unit

This unit is responsible for diagnosis of viral diseases applying patholgenicity test method and identification of different viruses. It is achieved with the inoculation of virological samples in embryonated chicken eggs through different approaches followed by HA/HI and AGID tests. The sources of samples of this unit are mainly post-mortem unit of CVL and a few from field condition.

Diagnosis of PPR is done by pen side test, which can be done in field condition. Occular and nasal swabs of goat and 1% piglets RBC are required for this test. This unit does have great contribution in the diagnosis of avian influenza. Rapid test was used for the purpose followed by virus isolation in the embryonated chicken eggs. Those samples were reconfirmed and verified by the OIE reference laboratories of Australia and Italy.

Progress

During 2062/63, a total of 958 samples from chicks, ducks and goats were tested for different viral diseases among which only 189 samples found positive. A total of 71 sample tentatively diagnosed as New Castle disease were received from Kathmandu, Biratnagar, Nuwakot, Gorkha, Pokhara and Kanchanpur district of which 34 samples were found positive as shown in table 12.

Table 12 : Test results of New Castle disease

S. N.	District	Species	No. of sample tested	No. of sample positive
1.	Kathmandu	Avian	38	26
2.	Biratnagar	Avian	05	00
3.	Kanchanpur	Avian	07	05
4.	Gorkha	Avian	01	00
5.	Nuwakot	Avian	05	03
6.	Pokhara	Avian	05	00
Total			**71**	**34**

Similarly, a total of 127 serum samples received from different Chitwan and Kathmandu district were tested for New Castle disease applying HI method. Out of them, 123 samples were found positive as shown in table 13.

Table 13 : Results of New Castle disease by HI method

S. N.	District	Species	No. of sample tested	No. of sample positive
1.	Chitwan	Avian	120	116
2.	Kathmandu	Avian	07	07
Total			**127**	**123**

A total of three samples suspected for equine influenza were received from Udaypur district and examined through egg inoculation method which showed none of the samples positive. The result has been shown in table 14.

Table 14 : Test Results of Equine Influenza

District	Species	No. of sample tested	No. of sample positive
Udaypur	Equine	3	-

Similarly, a total of four samples suspected for pox received from Dholkha district were examined through egg inoculation method which revealed non-occurrence of the disease as shown in table 15.

Table 15 : Test result of buffalo pox

District	Species	No. of sample tested	No. of sample positive
Dholkha	Buffalo	4	-

A total of 71 samples were tested for avian influenza through egg inoculation method which revealed none of the samples positive for its occurrence as shown in table 16.

Table 16 : Test Results of Avian Influenza by Egg Inoculation method

S. N.	Species	No. of sample tested	No. of sample positive
1.	Poultry	48	-
2.	Pigeon	3	-
3.	Wild bird	6	-
4.	Laucat	1	-
5.	Chicken Soup	8	-
6.	Dog Food	4	-
7.	Turkey Meat	1	-
	Total	**71**	**-**

Besides, this unit also received 65 samples from various district of the country namely; Kathmandu, Latitpur, Dhanusha, Ramechhap, Kailali and Kaski for the diagnosis of PPR. These samples were tested through penside test. A total of 30 samples were found positive and the result has been shown in table 17.

Table 17 : Examination of PPR by penside test method

S. N.	District	Species	No. of sample tested	No. of sample positive
1.	Kathmandu	Goat	26	12
2.	Lalitpur	Goat	10	10
3.	Janakpur	Goat	14	3
4.	Ramechhap	Goat	4	4
5.	Pokhara	Goat	3	-
6.	Kailali	Goat	8	1
Total			**65**	**30**

2.3 Rabies diagnosis unit

One of the most important works done during 2062/063 in this unit is diagnosis of rabies. Although it is the part of virology, various test related to diagnosis of this disease are performed separately than others because of public health importance. Various diagnostic tests conducted for rabies diagnosis include Negri body test, fluorescence antibody test and biological test.

A total of 24 samples were received from field condition comprising12 cases from dog and nine samples from cow, and one each from goat, mouce and cat. Among them, 17 cases were diagnosed as positive through all the aforementioned tests. Detail of the test results is given in table 18.

Table 18 : Result of different tests for diagnosis of rabies

S. N.	Test Conducted	Species	No. of sample tested	No. of sample positive
1.	NB test/FAT/ Biological test	Canine	12	8
2.	NB test/FAT/ Biological test	Bovine	9	7
3.	NB test/FAT/ Biological test	Caprine	1	1
4.	NB test/FAT/ Biological test	Feline	1	-
5.	NB test/FAT/ Biological test	Mice	1	1
	Total		**24**	**17**

Biochemistry Unit

1. Introduction

The Biochemistry unit of CVL mainly deals with the analyses of urine and serum samples. The samples are directly brought by the farmers, received from DLSOs and RVLs, and also collected from field during investigation of epidemics. These samples are processed in this laboratory unit based on the standard operating protocol of the unit and other protocols as per need. Following tests are done for biochemical analysis.

1. Calcium estimation
2. Phosphorous estimation
3. Zinc estimation
4. SGOT, SGPT
5. Protein and albumin estimation

2. Biochemistry unit

Urine samples are studied and analysed with the use of following techniques.

1. Commercial kits (Multistix/Uristix)
2. Microscopic examination

Urine sample is examined for specific gravity, sugar, ketone bodies, albumin, bilirubin, triple phosphate, calcium oxalate, RBC, pus cells etc. A total of one hundred and twenty urine samples comprising of 55 samples from canine and rest from cattle were tested during F/Y 2062/063. The urine samples of cattle received were mostly requested to rule out haematuria while the same from canine were tested against diabetes, urinary caliculi, kidney function impairment, jaundice, ascites, and haemoglobinuria. The result of microscopic examination of urine showed, in most of the cases, the presence of pus cells and calcium oxalate. Table 1 shows vatious types of biochemical estimations of sera of dogs and cattle.

3. Haematology Unit

The responsibility of this unit is to analyze the different parameters of whole blood samples collected in ethylene diamine tetraacetic acid and blood smear received from DLSOs, RVLs, central veterinary hospital and those collected during disease out breaks. Similarly the serum samples were analysed for phosphorous, calcium, zinc, total protein and glucose estimation of 606 samples are presented in Table No 1.

Specimen of whole blood is studied for haemoglobin content, packed cell volume, total erythrocyte count, total leukocyte count, differential leukocytes count, total platelets count and erythrocyte sedimentation rate.

Five hundred and eighty-seven blood samples were studied for the aforementioned parameters during F/Y 2062/63 among them, 104 samples comprising 93 from canine, two from equine and nine from cattle were also examined for the occurrence of blood protozoan parasites. Of themj, 31

samples were positive for *Babesia* spp, six for *Anaplasma* spp and only one sample for *Trypanosoma* spp.

Table 1 : Vatious types of biochemical estimations of sera of dogs and cattle

S. N.	Sample tested	No. of samples	Dog	Cattle
1	Calcium estimation	124	-	124
2	Phosphorous estimation	132	-	132
3	Glucose estimation	49	49	-
4	Zinc	135	-	135
5	Total Protein	46	46	
	Total	**486**	**95**	**391**

Table 2 : Parameter of tested samples of various species

S. N.	Species	Total	Hb	PCV	ESR	TLC	DLC	Blood protozoa
1	Cattle	188	55	28	35	35	26	9
2	Buffalo	138	55	24	16	15	28	-
3	Dog	198	33	32	13	15	12	93
4	Horse	63	22	10	10	8	11	2
	Total	**587**	**165**	**94**	**74**	**73**	**77**	**104**

Parasitology Unit

The Parasitology unit is involved in routine examination as well as investigation of different digestive tract parasites and non-digestive tract endoparasites of animals and birds causing adverse effects on livestock and poultry health as well in production. Faecal samples, skin scrapings, blood samples from different animals and birds are examined by adopting standard test protocols. They are done mainly for identification of eggs/ovas of different nematodes, cestodes, trematodes and other common parasites found in gastrointestinal system of livestocks.

Table : Results of various types of parasitological examination (2062/063)

S. N.	Type of Parasites	Species	4	5	6	7	8	9	10	11	12	1	2	3	Total
1	*Fasciola* spp	Cow	22	10	23	25	32	45	56	53	67	30	15	23	401
		Bull	1	-	-	-	-	-	-	-	-	-	-	-	1
		Goat	7	6	25	20	45	76	43	10	54	12	10	12	320
		Sheep	-	-	-	1	-	-	-	-	-	-	-	-	1
		Buffalo	40	34	32	64	30	25	40	57	40	50	86	65	563
		Dog	-	-	-	-	-	-	-	-	-	-	-	1	1
2	*Paramphistomum* spp	Cow	17	7	2	1	-	9	2	12	-	-	-	-	50
		Goat	1	-	-	1	-	-	-	-	-	-	-	-	2
		Buffalo	2	-	-	-	-	-	-	-	-	-	-	-	2
		Sheep	-	-	-	1	-	-	-	-	-	-	-	-	1
		Monkey	-	-	-	-	-	-	-	1	-	-	-	-	1
3	Strongyles	Cow	11	11	-	1	-	-	-	12	-	-	-	-	35
		Goat	19	22	43	23	31	12	16	1	10	18	20	24	239
		Buffalo	2		-	-	-	-	-	9	-	-	-	-	11
		Pig	-	10	-	-	-	-	-	-	-	-	-	-	10
		Horse	-	-	-	-	-	7	-	-	-	-	-	-	7
		Monkey	-	-	-	-	-	-	-	29	-	47	90	40	206
4	Skin scrapping	Dog	8	-	-	-	-	-	-	3	-	-	-	-	11
5	Blood protozoa	cow	-	2	-	-	-	-	-	-	-	-	-	-	
		Dog	-	-	-	-	-	-	-	1	-	-	-	-	1
6	Coccidia	Poultry	-	-	-	-	-	-	-	-	-	-	-	-	
7	Ascasids	Pig	-	7	-	-	-	-	-	-	-	-	-	-	7
		Dog	-	-	1	-	-	-	-	-	-	-	1	-	2
Positive			130	109	126	156	138	174	157	181	171	157	221	165	1885
Negative			54	60	90	123	120	124	120	201	90	145	109	129	1365
Total sample			184	169	216	279	258	298	277	382	261	302	330	294	3250

(Note : The numbers in the head of the table is indicative of Nepalese fiscal years. The digit 4 represents Shrawan and so forth.)

Qualitative test is done by the technique of double floatation for detection and identification of the eggs of gastrointestinal parasites whereas the quantitative test is performed by the modified Mc Master's counting methods for the determination of number of eggs per grams in the feces which helps in the evaluation of the extent of parasitic burden in a particular animal species. Furthermore, this unit also carries out larvae culture for the identification of nematodes. Similarly, skin scrapings for the presence of mites, blood samples for the presence of blood parasites are routinely carried out. All these laboratory works are being conducted in collaboration with RVLs and animal health research division of Nepal agriculture research. In addition, this unit is also involved in the surveillance of parasitic infestations in various wild and zoo animals regularly since past few years.

Samples from districts, private practitioners are also being examined too asses the magnitude of parasites and parasitism. Since last few years this unit is actively involved in collaborative research work and study programme of graduate and post- graduate study of Trivuwan University and Purwanchal University in field of Parasitology. Test result of parasitological examination has been presented in following table. Over the last fiscal year 3250 samples of cattle, buffaloes, goats, dogs, poultry and monkeys were tested out of which 1885 were found positive for parasites. The various types of parasitological examination conducted during the F/Y 2062/063 have been presented in tabular from as shown below.

Molecular Biology Unit

1. Introduction

Molecular techniques are very sensitive, fast and reliable tools for disease diagnosis and research works. Therefore, molecular biology unit of central veterinary laboratory has started to diagnose the bacterial and viral diseases by extracting DNA and RNA with the use of polymerase chain reaction (PCR) test and reverse transcriptase polymerase chain reaction (RT-PCR) techniques.

2. Activities and progress

During F/Y 2062/2063, a total 26 samples were tested among which 21 samples were tested for highly pathogenic avian influenza (H5N1)virus and five samples were tested for haemorrhagic septicemia (HS).

For the detection of HS, samples of two bacterial cultures, two bone marrow and one tissue swab were collected from Kathmandu Valley. Firstly, DNA was extracted from all the samples and then they were set in for PCR. During the PCR test, specific primers namely KMT1SP6 (F) forward and KMT1T7(R) reverse primers were used. The PCR product was subjected to electrophoresis using 2% agarose gel for one hour and then observed under fluorescence. The two bacterial culture colonies showed positive bands at 460 base pair but rest samples didn't give any band at the same base pair. From the result, therefore, two samples were found to be positive for HS and rest were found to be negative.

On the other hand, Reverse Transcriptase Polymerase Chain Reaction (RT- PCR) test method has been performed to detect H5N1 virus from F/Y 2062/063 at CVL. A total 21 poultry samples comprising 18 sera collected from different districts; Chitwan, Kanchanpur, Dhangadhi, and three samples of allantoic fluid collected from inoculated eggs in virology laboratory unit of CVL were tested. For the test, firstly RNA was extracted from above samples by using commercial kits (QIAamp® Viral RNA Mini Kit) according to the manufacture's instruction. One-step RT-PCR was performed for both cDNA synthesis and PCR amplification in a single tube using gene-specific primers (Recommended by WHO H5 Reference Laboratory Network).

The PCR product so prepared was subjected to electrophoresis using 2% agarose gel and observed under ultraviolet light in which the samples showed no positive band of HPAI H5N1 virus at 219 base pair and therefore, all the samples were negative.

National Avian Laboratory
Bharatpur

1. Introduction

National avian laboratory (NAL) is situated in Bharatpur of Chitwan district. It looks after the various diseases of birds especially the commercial poultry. To deliver various test results, NAL is provided with Pathology (includes Post mortem and Histopathology), Microbiology (includes Bacteriology, Mycology and Virology) and Washing & sterilization units.

2. Programme and progress

2.1 Annual Programme & Progress, NAL, Bharatpur (2062/063)

S.N.	Activity	Unit	Target	Budget	Progress	Progress %
1	**Diagnostic Services**					
1.1	Pathology	Number	2100	57000.00	2110	100
1.2	Histopathology	„	600	129000.00	607	100
1.3	Microbiology	„	1000	86000.00	1007	100
1.4	Biochemical	„	300	25000.00	219	73
1.5	Virology	„	300	75000.00.	302	100
1.6	Serology	„	300	83000.00	307	100
2	**Laboratory maintenance**					
2.1	Lab water system	Times	1	2500.00	1	100
2.2	Lab animal	„	1	2800.00	1	100
2.3	Cold chain		1	3300.00	1	100
3	**Investigation & surveillance of Avian Disease**					
3.1	Epidemic Investigation	„	3	135000.00	3	100
4	**Reporting & Publication**					
4.1	Annual Technical Report	„	1	5000.00	1	100
4.2	Browser	„	1	10000.00	1	100
4.3	Epidemic Reporting	„	12	4000.00	12	100
5	**Purchase**					
5.1	Technical book & journals	„	1	40000.00	1	100
5.2	Computer & printer for ELISA	„	1	50000.00	1	100
5.3	Lab furniture	Number	1	25000.00	1	100
5.4		Time	1	25000.00	1	1007
6	**Workshop & Training programme**					
6.1	Avian disease investigation.	„	1	40000.00	1	100

2.2 Human resource situation, NAL, Bharatpur (062-063)

S.N.	Type of the post	Class	Number	Fulfilled	Vacant
A	**Technical**				
1	Senior Veterinary Officer	Gazetted II	1	1	-
2	Veterinary Officer	Gazetted III	2	2	-
3	Vet. Technician	Non-gazetted (NG) I	2	2	-
4	Junior Vet. Technician	NG II	2	2	-
Total Technical staffs			7	7	-
B	**Administration/Account**				
1	AsstistantAccountant	NG II	1	1	-
2	Clerk	NG II	1	1	-
3	Peon	-	2	1	1
Total Administrative staff			4	3	1
Grand Total			11	10	1

2.3 Description of human resource, NAL, Bharatpur (2062-063)

S.N.	Designation	Post	Class	Remarks
1	Dr. Tika Ram Neaupane	SVO	G.II	
2	Dr. Daya Ram chapagain	VO	G III	
3	Dr.Peetambar S.Kushwaha	VO	G III	On leave
4	Mr.Shailendra Bhandari	VT	NG I	
5	Mr.Endu Raya Yadav	VT	NG.I	
6	Mr.Ram Pd. chaudhari	JVT	NG II	
7	Mr. Binod kumar Saphy	JVT	NG II	
Administration/Account				
8	Mr. Krishna kumar Maharjan	Asstistant Accountant	N GII	
9	Mr.Rishee Ram Acharya	Clerk	NG II	
10	Mr.Bhanu vakt Sapkota	Peon	Lower level	
11	Mr.Purna Prasad Sapkota	Peon	Lower level	

3. Laboratory services

3.1 Pathology

Necropsy examination of dead poultry birds are carried out for intial study of the disease processes. Based on the tentative diagnoses, various types of specimens are collected and delivered to other laboratory units of NAL for the purpose of confirmative diagnosis. The trend of disease occurrence among commercial poultry during the F/Y 2062/063 has been presented in table 1 below.

Similarly, a total of six hundred and eight samples were received at histopathology unit during 2062/063. Among them, 200 samples were diagnosed as the case of Marek's disease followed by 150 samples for infectious bursal disease, 78 samples for gout, 50 samples each for New Castle disease and leukosis, 25 samples each for hydropericarditis and toxicity and 20 saples for fatty liver syndrome.

Table 1 : Trend of disease occurrence in commercial poultry (2062/063)

S.N.	Disease	Total cases
1	Collibasilosis	70
2	Mycotoxicosis	35
3	Pullorum disease	33
4	New Castle disease	30
5	Coccidiosis	23
6	Enteritis	22
7	Immune Supression	19
8	Mycoplasmosis	19
9	Infectious bursal disease	14
10	Intestinal helminths	12
11	Hydropericardium syndrome	9
12	Avian infectious bronchitis	8
13	Fowl cholera	8
14	External parasites	7
15	Marek's disease	7
16	Ascitis	6
17	Omphalitis	4
18	Fowl typhoid	3
19	Infectious coryza	3
20	Peritonitis	3
21	Lymphoid leukosis	2
22	Fatty Liver Syndrome	1
23	Hemorrhagic Syndrome	1
24	Metabolic Disorder	1

3.2 Microbiology

Of the one thousand and seven samples received, 535 cases were subjected to drug sensitivity test to find out the effective drugs. On the bacteriological culture, colibascillosis, salmonellosis, pasteurallosis, staphylococcosis were found and tested for antibiotic efficacy. Sensitivity of different antibiotics was ciprofloxacin- 93%, chloramphenical-67%, enrofloxacin-89%, gentamycin-57%, tetracycline-44%, cotrimoxazole- 2% and amoxicillin- 3%.

3.3 Virology

Only one pathogenicity test, the egg inoculation, is conducted for the provisional diagnosis of New castle disease, Marek's disease and infectious bursal disease. Altogether three hundred and two specimens were subjected to egg during 2062/063.

3.4 Serology

In serology unit, the following diagnostic tests were performed. Specimens that could not be diagnosed at NAL were referred to CVL. The various diagnostic tools in serology unit are as follows. During 2062/063, 218 sera were tested through HA/HI technique, 30 cases through Immunocomb kit, 307 cases through plate/slide agglutination for the diagnosis of various diseases.

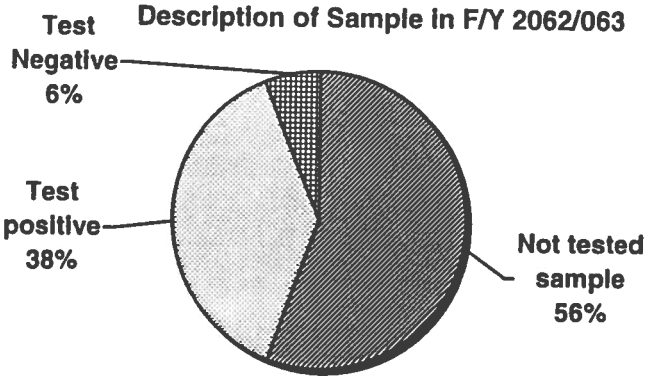
Test methods for different diseases of poultry

The diiferent diagnostic methods adopted in serology unit are simple agglutination (includes slide and plate agglutination), haemagglutination, haemagglutination Inhibition,, immunocomb (for infectious bronchitis, infectious bursal disease, and New castle disease, Agar gel precipitation test and rapid test for Avian influenza.

Biochemical analysis of serum

Different serum samples are analysed for the estimation of calcium, phosphorus and total protein. A total of 219 sera were subjected for biochemical analysis.

The following pie- diagram shows the status of different samples during F/Y 2062/063.



Regional Veterinary Laboratory
(Eastern Region)

1. Introduction

Regional veterinary laboratory (RVL) of eastern development region is situated in sub-metropolitan city, Biratnagar-17. Although established in 1988-89 AD, diagnostic activity of the laboratory was possible from f/y 1990-91. Insufficient number of workforce, poor availability of necessary equipments and unstable government policy was few causes behind it. More over, RVL got separate identity in Department of Livestock Services in 1991-92. The domain of activities of the eastern laboratory covers sixteen districts under three zones namely; Mechi, Koshi and Sagarmatha.

2. Programme and progress

Annual programme and progress, RVL, Biratnagar (2062/063)

S.N.	Programme	Unit	Target	Progress	Weight	% Progress
1	**Laboratory Services**					
1.1	Parasitological examination	Number	1000	1932	4.38	100
1.2	Microbiological examination	Number	400	806	7.5	100
1.3	Pathological examination	Number	200	314	2.5	100
1.4	Serological examination	Number	500	701	6.25	100
1.5	Haematological examination	Number	300	314	3.75	100
1.6	Biochemical examination	Number	400	1074	7.5	100
1.7	Sample sent to other laboratories	Number	200	1114	2.5	100
2	**Investigation and Surveillance Program**					
2.1	Study of infertility (repeat breeding) in cattle	Times	6	6	18.5	100
2.2	Sukhadia investigation	Times	6	6	17.5	100
2.3	Epidemic investigation	Times	6	6	17	100
3	**Supervision & monitoring of District Livestock Service Offices**	**Times**	**6**	**6**	**3.75**	**100**
4	**Animal Disease Investigation Workshop**	**Times**	**1**	**1**	**3.88**	**100**
5	**Publication and Epidemic Reporting**					
5.1	Epidemiological bulletin	Times	4	4	2.5	100
5.2	Annual technical report	Times	1	1	.63	100
6	**Purchase**					
6.1	Scientific and technical books	Times	1	1	1.88	100

3. Laboratory Services

3.1 Diagnostic Services

The routine laboratory work includes examination of faeces, milk, skin scrapings, urine, sera and blood to obtain test results intended for various types of use. Besides, it includes seromonitoing of a few infectious diseases. Faecal samples and skin scrapings are tested using various techniques to explore the presence of different types of digestive and non-digestive tract parasites. Specimen of milk is screened for the occurrence of Mastitis through CMT and MWT tests. The positive samples are then cultured to isolate and identify the etiological agent. Similarly blood and urine samples are analyzed using clinical pathology techniques. These include estimation of haemoglobin (Hb), packed cell volume (PCV), total blood cell count (TC), differential leukocyte count (DLC), calcium, phosphorus, and total protein, and exanimation of blood protozoa. Serological test is done to screen Brucellosis in farm animals and salmonella in poultry and hypersensitivity test (tuberculin test) is done to screen tuberculosis in animals.

3.1.1 Parasitology

Various types of digestive tract and non-digestive tract parasites are examined identified, and their eggs are counted using both qualitative as well as quantitative testing procedures in Parasitology laboratory unit. Most of the fecal samples are received from farmers and district livestock service offices. However, the laboratory also collects faecal samples from field cases during surveillance and investigation programme. Qualitative examination of the fecal samples is done using sedimentation and floatation techniques. Quantitative examination is done as per demand through Mc Master Technique.

A total of 1932 faecal samples from different species of animal were received and examined during the fiscal year 2062/063. Among them, 1393 samples (72.1%) were positive and 539 samples (27.9%) negative. The result of faecal examination revealed that fascioliosis (52%) was the most prevalent parasitic infestation followed by Distomiasis especially paramphistomes (28.65%) and nematodiaisis. A detail of the faecal examination has been given in table 1.

Similarly, forty-one samples of skin scraping from different species of animals were received and examined for the occurrence of external parasites. Of them, 28 samples were negative and 13 samples were positive which includes nine cases of Sarcoptes and four cases of Psoroptes).

3.1.2 Haematology

Clinical pathology study of blood samples includes estimation of Hb and PCV, TEC, TLC and DLC. Besides, blood samples received from various districts were examined for blood parasites. Out of total number of 246 samples, only thirty-three samples were positive which includes 27 cases of Babesiosis and six cases of Theileriosis. The occurrence of Babesiosis, Theileriosis and Anaplasmosis over seven years has been presented in figure 1.

Table 1 : Month wise faecal examination (2062/063)

Mon	Total Sample	+ ve	- ve	L F	Pa	Co	Bun	Str	Oe	Ha	As	Tri	Others
July	174	100	74	46	26	5	1	10	4	1	4	1	2
Aug	203	160	43	62	39	7	10	9	15	2	0	2	14
Sept	257	214	143	105	78	4	8	7	6	0	2	2	2
Oct	146	113	33	44	47	2	10	4	2	1	1	0	2
Nov	139	99	40	49	29	4	6	0	6	0	0	0	5
Dec	102	74	28	39	23	3	2	0	4	0	1	0	2
Jan	98	77	21	36	24	2	0	8	6	0	1	0	0
Feb	143	113	30	59	38	1	1	1	8	0	0	0	5
Mar	117	91	26	58	23	3	0	0	3	1	1	0	2
April	111	83	28	48	21	3	5	0	0	2	0	0	4
May	183	142	41	95	29	6	5	2	3	1	0	0	1
June	159	127	32	84	22	5	2	0	7	5	0	0	2
Total	**1932**	**1393**	**539**	**539**	**399**	**45**	**50**	**41**	**64**	**13**	**10**	**5**	**41**

Note :(LF : Liver fluke, Pa : Paramphistomes, Co : Coccidia, Bun : Bunostomum, Str : Strongyles, Oe : Oesophagostomum, Ha : Haemonchus, As : Ascarids, Tri : Trichuris)

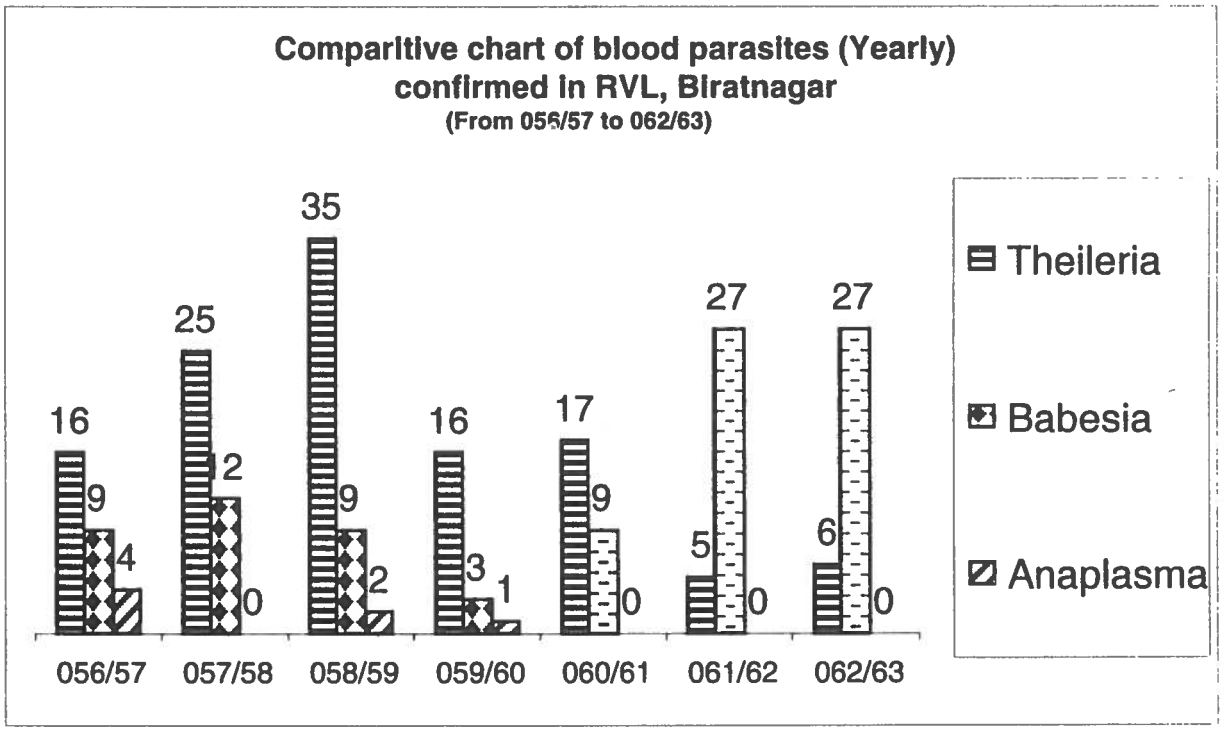


Figure 1 : Occurrence of Babesiosis, Theileriosis and Anaplasmosis over seven years

3.1.3 Microbiology

Microbiology laboratory unit received a total number of eight hundred and six milk samples of which 412 samples were screened as positive at CMT and MWT tests. The most prevalent bacterial isolates were *Staphylococcus, Streptococcus, E. coli, Klebsiella, Pseudomonas* and *Enterobacter*

spp etc. The month wise screening of milk samples for the occurrence of Mastitis is given in table 2 and the result of antibiotic sensitivity test has been shown in the figure 2.

Table 2 : Month wise screening of milk samples for the occurrence of Mastitis

Months	Total sample	CMT/MWT (+ve)	CMT/MWT (-ve)	Percent positive
July	70	41	29	59
August	94	54	40	58
September	117	72	45	52
October	25	13	12	59
November	45	26	19	58
December	40	26	14	65
January	124	18	106	15
February	31	19	12	61
March	117	39	78	34
April	25	14	11	56
May	48	43	5	90
June	70	47	23	68
Total	**806**	**412**	**394**	**51**

3.1.4 Pathology

Majority of the cases of necropsy examination includes poultry and only occasionally a few case includes animals. Impression smears, swab, tissues are collected at post mortem. Organs/tissues that are collected for histological examination are sent to central veterinary laboratory. Reports of post mortem examination of 314 cases of poultry suggests 50% cases of infectious bursal disease, 15% cases of coccidiosis 10% cases each of New Castle disease and internal parasites, 5% cases of chronic respiratory disease, 4% cases of sudden death syndrome and 6% includes diseases of various etiology.

3.1.5 Biochemistry

Serum and urine are the chief specimens with which various types of biochemical estimation and analyses are done. Serum samples are collected from different farm sheds and sites of investigation programme of the laboratory. Altogether 1074 serum samples were collected and analyzed for the estimation of total protein, glucose, phosphorus, zinc using commercial kits. Similarly, analysis of urine was done for specific gravity, pH, sugar, albumin, ketone bodies, urobilinogen and protein using dipsticks (multisticks) for most of the test purpose. However, other biochemical estimation methods are also applied, for example; Rothera's test is used for ketone bodies and Robert's test for the detection of protein.

3.1.6 Serology

Serological examination is done mainly for two diseases namely salmonellosis and brucellosis. A total of 204 samples were tested for pullorum disease by Plate agglutination method (PAT) among which only two sample were positive. Similarly, Rose Bengal plate test (RBPT) is done for

screening the Brucella positive animals. This test was done with serum samples collected from 297 cattle and buffalo and all the samples were negative. Similarly, Tuberculin test is done in cattle and buffalo for the occurrence of tuberculosis. No individual was found positive among seven animals tested so far.

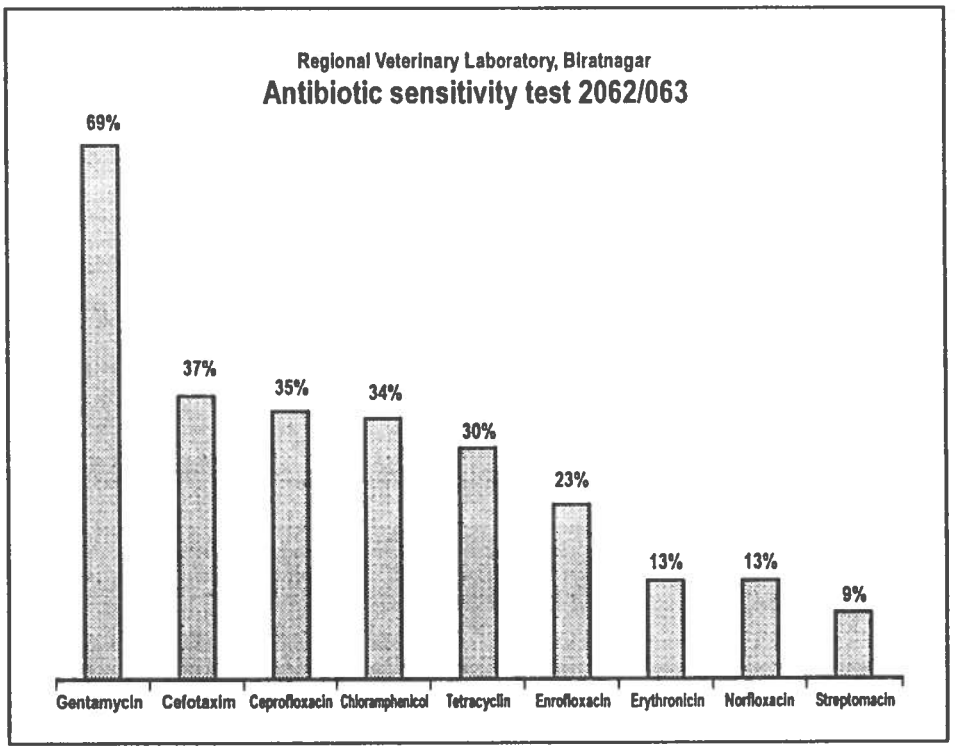


Figure 2 : Efficacy of various antibiotics

3.2 Other Services

3.2.1 Sample sent to other laboratories

Samples that are not possible to examine at RVL, Biratnagar are sent to other laboratories. Majority of such samples are sent to CVL as it is reference veterinary laboratory in the country. However, a few samples are also sent to national TADs and FMD laboratory, Kathmandu. Sometimes, bacterial growths are dispatched to CVL for reconfirmation of the test results. A detail of the dispatch of samples has been given in table 3.

Table 3 : Details of samples dispatched to CVL

S.N.	Type of samples	Number	Remarks
1	Bacterial culture	25	Reconfirmation
2	Swab	20	
3	Organs/tissues	5	Histological test
4	Cyst	11	Parasite identification
5	Lantana Plant	4 times	Proximate analysis
6	Serum	548	
7	Avian influenza surveillance	566	
	Total	1179	

3.2.2 National PPR programme

The total number of dose of PPR vaccines received from Directorate of animal health was one hundred thousand among which 71,000 doses of vaccines were distributed as shown in table 4 and the status of PPR in eastern development region has been shown in figure 3.

Table 4 : Distribution of PPR vaccine

S. N.	Districts	Doses
1	Morang	20,000
2	Jhapa	12,000
3	Sunsari	5,000
4	Udaypur	11,000
5	Saptari	500
6	Siraha	22,500
Total		**71,000**

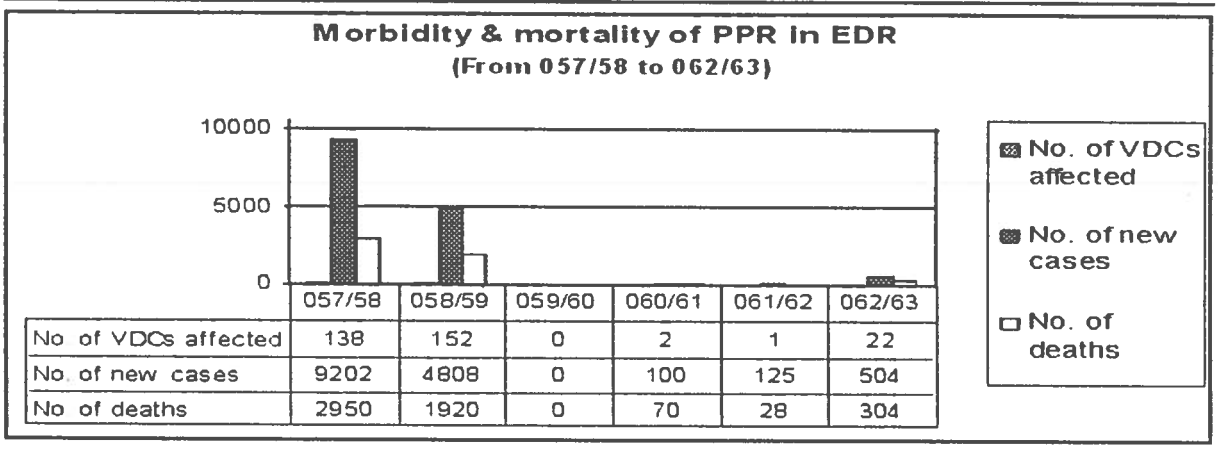
The details of the activities under national PPR surveillance programme has been given in table 5.

Table 5 : National PPR sero-monitoring programme

DLSOs	Udaypur	Sunsari	Morang	Siraha	Saptari	Dhankuta	Illam	Jhapa	Total
Vaccination to be done (No.)	24000	24000	24000	24000	24000	24000	24000	24000	180000
Serum to be collected (No.)	120	120	120	120	120	120	120	120	900
Serum collected	125	129	122	120	120	75	125	122	938

3.2.3 Bird Flu surveillance

Surveillance of bird flu has been started at CVL since 2003. Earlier, active surveillance was confined to a few locations. When the disease was reported in India early in 2006, CVL initiated nation wide surveillance programme particularly in the areas of Indo-Nepalese border. RVL, Biratnagar got responsibility of sample collection in eastern development region. In F/Y 2062/063, surveillance programme was done in all the districts of the region. Serum samples and swabs were collected from both wild birds, ducks, pigeons and poultry which includes commercial and backyard poultry and poultry from live bird market. Surveillance activities were also concentrated in the area of Koshi Tappu which is considered one of the popular habitats for migratory birds in Nepal. These samples were sent to CVL for testing against bird flue. The detail information on the types of birds and areas where sample collection was done is given in table 6 and 7.



	057/58	058/59	059/60	060/61	061/62	062/63
No of VDCs affected	138	152	0	2	1	22
No of new cases	9202	4808	0	100	125	504
No of deaths	2950	1920	0	70	28	304

Figure 3 : Status of PPR in eastern development region

Table 6 : Samples received from different DLSOs/Quarantines

S. N.	Office	Type of specimen			Remarks
		Dead bird	Serum	Swab (Tr/Cl)	
1	DLSO, Sunsari	3	-	-	CVL
2	DLSO, Illam	-	10	-	CVL
3	DLSO, Jhapa	-	10	10	CVL
4	Animal Quarantine Office (AQC), Biratnagar	-	20	20	CVL
5	AQC, Kakarbhitta	-	52	10	CVL
	Total	**3**	**92**	**40**	

Table 7 : Sample collected by RVL, Biratnagar

S.N.	Location	Type of birds	Type of sample				Remarks
			Dead bird	Serum	Swab		
					Tracheal	Cloacal	
1	Purbanchal poultry farm, Saptari	Layer	-	30	15	18	Sample sent to CVL
2	Koshi Tappu Banya Jantu Aaraksha, Sunsari	Sarash	2	-	-	-	Sample sent to CVL
		Hoopoe	1	-	-	-	
3	V.D.C. Rangeli, Morang	Poultry	1	-	-	-	Sample sent to CVL
		Pigeon	1	-	-	-	
		Rupi	1	-	-	-	
		Jureli	1	-	-	-	
4	V.D.C. Rangeli, Sanischare, Morang	Pigeon	1	-	3	3	Sample sent to CVL
		Crow	1	1	-	-	
		Poultry	-	-	2	2	
		Pig	-	1	1	2	
		Duck	-	-	1	1	

5	V.D.C. Belbari, Biratnagar-1,6,8,9 (Rani border area)	Broiler	-	134	88	61	Sample sent to CVL
6	District : Dhankuta	Broiler	-	9			Sample sent to CVL
7	Jhapa (Kakarbhitta, Surunga, Topgachi, Damak)	Broiler	-	40	10	-	Sample sent to CVL
Total			**9**	**215**	**120**	**87**	

3.2.4 Vaccination

RVL, Biratnagar provided phrophylactic services in the EDR, details of which has been shown in table 8 below.

Table 8 : Phrophylaxis in EDR (2062/063)

Month	Black quarter	Haemorrhagic septicaemia	Anthrax	Swine fever	PPR	Foot & mouth disease	Rabies	Total
July	680	5313	541	-	-	-	141	6675
August	1666	10331	-	204	-	-	907	13058
September	3376	4483	-	40	6000	-	563	14462
October	3441	4086	-	499	2400	150	89	10665
November	1493	1539	-	6722	-	-	189	9943
December	8029	8004	-	300	21500	40	102	37975
January	3385	4091	-	2	-	300	131	7909
February	5654	6184	-	507	1200	-	619	14164
March	6824	8130	-	507	6650	346	196	22653
April	4556	6786	-	400	34452	-	178	46372
May	10414	7270	-	-	52210	-	98	69992
June	13910	11285	-	98	46157	221	108	71779
Total	**63428**	**77502**	**541**	**9279**	**170569**	**1057**	**3321**	**325697**

Regional Veterinary Laboratory
(Central Region)

1. Introduction

Regional veterinary laboratory of the central development region is situated in Janakpur that provides diagnostic services to all the 19 districts of the region. Various diseases diagnosed at the laboratory is achieved through its various laboratory units; pathology, parasitology, microbioliogy; haematology and biochemistry. Serological and histopathological laboratory test results are obtained by dispatching the relevant specimens to CVL as these diagnostic facilities are not available in this laboratory.

2. Programme and progress

Programme and progress, RVL, Janakpur (2062/063)

S. N.	Programs and activities	Annual target			Annual Progress	
		Unit	Target	Weightage (%)	Progress	Weightage (%)
1	**Laboratory Services**					
1.1	Parasitological Examination	Number	100	5/232	178	5/232
1.2	Microbiological Examination	Number	300	6/726	305	6/726
1.3	Pathological Examination	Number	250	3/737	254	3/737
1.4	Serological Examination	Number	300	4/484	315	4/484
1.5	Hematological Examination	Number	450	6/726	454	6/726
1.6	Biochemical Examination	Number	250	5/531	290	5/531
1.7	Sample collection and dispatch	Number	200	2/99	227	2/99
2	**Disease investigation and surveillance**					
2.1	Investigation of external & internal parasites of claves	Times	6	27/95	6	27/95
2.2	Investigation of Epidemics	Times	6	19/43	6	19/43
3	**Monitoring and supervision**					
3.1	Monitoring & supervision of district labs of DLSOs	Times	6	5/979	6	5/979
4	**Epidemiological Reporting & publication**					

4.1	Quarterly Epidemiological Bulletin publication	Times	4	3/737	4	3/737
4.2	Annual technical report publication	Times	1	0/747	1	0/747
5	**Technical workshop**					
5.1	Technical workshop on disease investigation	Times	1	5/232	1	5/232
6	**Purchase Program**					
6.1	Purchase of scientific books	Times	1	1/495	1	1/495

3. Laboratory Services

3.1 Parasitology

3.1.1 Coprological examination

In the F/Y 2062/063 altogether 178 Faecal samples from different species of animals such as cattle, buffalo, goats, poultry and dogs were examined. Qualitative Feacal examination of 153 and EPG counts of 25 Feacal samples were conducted. Fasciola, Paramphistomum, Strongyle, Trichuries were major internal parasites identified. Detail description of results of monthly feacal examination during 2062/063 is presented in table 1.

Table 1 : Result of monthly feacal examination F/Y 2062/063

Parasites	Months (As per Nepalese Fiscal Year)												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Fasciola	1	1	2	2	2	3	1	-	-	-	3	-	15
Paramphistomum	10	8	6	3	5	6	5	6	9	7	5	10	80
Strongyles	4	2	4	3	-	-	2	-	1	3	2	6	27
Trichuris	2	1	-	-	-	1	-	-	1	2	-	-	7
Mixed infection	3	1	2	-	3	-	2	1	-	-	1	1	14
Total +ve	**20**	**13**	**14**	**8**	**10**	**9**	**10**	**7**	**11**	**12**	**11**	**17**	**143**
Total -ve	**2**	**-**	**1**	**-**	**-**	**2**	**6**	**4**	**5**	**3**	**12**	**-**	**35**

3.1.2 Skin scrapping examination

Thirty one skin scrapings from buffaloes, buffalo Calf, Ox and Goat were found positive for different external parasites. Out of that, 9 were from buffaloes, 10 from buffalo calves, 5 from Ox and rest 7 from goats. The detail of the skin scrapping test result is given in table 2.

3.2 Microbiology

A total of 305 samples were received for bacteriological examination among which 181 samples were found positive for different species of bacteria and the rest samples were found negative and details of the tested samples has been present in table 3. Similarly, efficacy of different antibiotics has been shown in table 4.

Table 2 : Test result of sking scrappings

Month	Total Sample	Species of Animals				Parasites (Species)
		Buffalo	Buffalo calf	Ox	Goat	
Shrawan	2	1	1	-	-	Sarcoptes
Bhadra	3	-	-	1	2	Sarcoptes Psoroptes
Ashoj	1	1	-	-	-	Sarcoptes
Kartik	2	1	1	-	-	Sarcoptes
Mansir	3	1	1	-	1	Sarcoptes
Poush	2	-	2	-	-	Sarcoptes Psoroptes
Magh	2	-	1	1	-	Sarcoptes
Falgun	3	1	1	-	1	Sarcoptes
Chaitra	4	1	1	1	1	Sarcoptes Psoroptes
Baishakh	5	2	1	2	-	Sarcoptes
Jestha	2	1	-	-	1	Sarcoptes
Ashadha	2	-	1		1	Sarcoptes
Total	**31**	**9**	**10**	**5**	**7**	

Table 3 : Monthly receipt of samples at microbiology unit

Duration	No. of Samples	Positive	Negative	Percentage(+ve)
Shrawan	5	3	2	60
Bhadra	20	16	4	80
Ashoj	24	15	9	62.5
Kartic	52	45	7	86.54
Mansir	12	7	5	58.33
Poush	20	12	8	60
Magh	30	19	11	63.33
Falgun	40	30	10	75
Chaitra	26	5	21	19.23
Baishakh	28	10	18	37.71
Jestha	30	4	26	13.33
Ashadha	18	15	3	83.33
Total	**305**	**181**	**124**	**59.34**

Table 4 : Antibiotic sensitivity test result

Antibioptics used	Percent efficacy
Enrofloxacin	80%
Ciprofloxacin	73%
Gentamycin	73%
Norfloxacin	68%
Ofloxacin	65%
Oxytetracyclin	50%
Amoxicillin	45%
Penicillin	25%

3.3 Haematology

A total of 454 blood samples were examined for different blood parameters as well as for blood parasites. Out of 454 samples, 409 blood samples were found Negative for any blood parasites & rest 45 were found positive for different blood parasites. The details are given in the following table No. 5

Table 5 : Details of blood sample examination

Month	Total Sample	Anaplasma	Babesia	Theileria	Tryps	- ve
Shrawan	7	0	-	-	-	7
Bhadra	30	2	2	-	-	26
Ashoj	2	1	1	-	-	-
Kartik	112	5	3	-	-	104
Mansir	20	2	2	-	-	16
Poush	25	-	-	-	-	25
Magh	40	-	-	-	-	40
Falgun	67	1	-	-	-	66
Chaitra	25	-	1	-	-	24
Baishakh	35	-	1	-	-	34
Jestha	40	-	4	-	3	33
Ashadha	51	6	6	-	5	34
Total	**454**	**17**	**20**	**-**	**8**	**409**

3.4 Pathology

The pathological examination includes a total number of 254 cases of post mortem examination were presented during the F/Y 2062/063. All the cases received were birds. No cases of large or small ruminants and other species of animals were received. It is mentioned that all the samples of birds received were brought from the Janakpur municipality and its adjacent areas. The status of poultry disease in the area is presented in table 6.

Table 6 : Trend of disease occurrence in poultry (2062-063)

S. N.	Tentative diagnosis	Total Cases	
		Number	Percent
1.	Coccidiosis	102	40.16
2.	CRD	83	21.68
3.	IBD	33	12.99
4.	Colisepticaemia	14	5.51
5.	Aflatoxicosis	12	4.72
6.	Miscellaneous (parasites, enteritis, mixed in infection)	7	2.76
7.	Salmonellosis	3	1.18
Total		**254**	**100%**

3.5 Serology

In this unit the serum samples are collected from different districts during disease investigation and surveillance program. Most of the serum samples collected from livestock and poultry were dispatched to CVL and other reference laboratories for diagnosis. Detail of the serological test results has been shown in table 7.

Table 7 : Serological test results

S.N.	Districts	Animal Spp.	No. of Sample	Test requested	Result		Remarks
					+ve	-ve	
1.	Dhanusha	Goat	92	PPR	20	72	sent to CVL
2.	Mahottari	Goat	52	PPR	13	39	sent to CVL
3.	Sarlahi	Goat	31	PPR	13	18	sent to CVL
4.	Bara	Goat	56	PPR	6	50	sent to CVL
5.	Ramechhap	Goat	9	PPR	-	-	sent to CVL
6.	Parsa	Buffalo	35	Brucellosis	-	-	not confirmed
7.	Dhanusha	Buffalo	45	Rinderpest	-	-	serosurveillance
8.	Mahottari	Pig	15	Swin Fever	-	-	not confirmed
9.	Dhanusha	Poultry	17	Bird flu	-	17	sent to CVL
10.	Mahottari	Poultry	11	Bird flu	-	11	sent to CVL
11.	Sarlahi	Poultry	18	Bird flu	-	18	sent to CVL
12.	Bara	Poultry	20	Bird flu	-	20	sent to CVL
13.	Parsa	Poultry	10	Bird flu	-	10	sent to CVL
14.	Makawanpur	Poultry	20	Bird flu	-	20	sent to CVL

Regional Veterinary Laboratory
(Western Region)

1. Introduction

Western Development Region (WDR) is situated between 82° 30' to 85° 15' east longitude and from 27° 15' to 29° 30' north latitude. It occupies about 20% (29355 Sq. Km.) of total areas of Nepal. The region shares boundaries with Uttar Pradesh of India in the south and Tibet of China in the north. The region is bulging between Central and Mid-western development regions of Nepal in the east and west respectively.

2. Programme and progress

2.1 Annual program and progress, RVL, Pokhara (2062/63)

S. N.	Programs and Activities	Annual Target			Annual Progress	Weightage %
		Unit	Target	Weightage (%)		
1	**Laboratory Services**					
1.1	Parasitological Examinations	Number	700	2.71	974	2.71
1.2	Microbiological Examinations	Number	400	6.51	552	6.51
1.3	Pathological Examinations	Number	400	4.34	791	4.34
1.4	Serological Examinations	Number	300	3.26	309	3.26
1.5	Hematological Examinations	Number	300	3.26	304	3.26
1.6	Biochemical Examinations	Number	200	3.26	226	3.26
1.7	Sample collection and dispatch	Number	400	4.34	703	4.34
2	**Disease Investigation & Surveillance Program**					
2.1	Mycoplasma disease investigation	Times	12	17.4	13	17.4
2.2	Investigation of Epidemic	Times	7	17.0	7	17.0
3	**Monitoring and Supervision**					
3.1	Monitoring and Supervision of district based Laboratories	Times	10	7.17	12	7.17
4	**Laboratory strenghening program**					

4.1	Investigation of bovine reproductive disorders	Times	12	17.6	12	17.6
5	**Publication Program**					
5.1	Tri-monthly Epidemiological Bulletin publication program	Times	4	2.17	4	2.17
5.2	Annual Technical Report Publication	Times	1	0.54	1	0.54
6	**Purchase program**					
6.1	Computer printer and freeze	Number	2	4.34	2	4.34
6.2	Postmortem table and lab rack	Number	4	1.63	4	1.63
6.3	Purchase of Scientific books	Number	4	1.08	4	1.08
7	**Workshop**					
7.1	Veterinary investigation workshop	Times	1	3.37	1	3.37

2.2 Description of human resource, RVL, Pokhara (2062/2063)

S. N.	Name of Staff	Post	Class	Post Sanctioned	Post Available	Vacant
1.	Dr. Vijay Chandra Jha	SVO	G II	1	1	0
2.	Dr. Shiva Prasad Devkota	VO	G III	1	1	0
3.	Miss Bishnu Kumari Basnet	JT	NG I			
4.	Mr. Yogendra Raj Regmi	JTA	NG II	1	1	0
5.	Mr. Bishnu Prasad Kafle	JTA	NG II	1	1	0
6.	Mrs. Til Kumari Sikdel	Typist	NG I	1	1	0
7.	Mr. Drona Prasad Adhikari	Kharidar	NG II	1	1	0
8.	Mrs. Gauri Dhungana	Sub-Accountant	NG II	1	1	0
9.		Stock man	NG III	2	0	2
11.		Driver (H.V.)	None	1	0	1
12.	Mr. Hari Bahadur Gharti	Peon	None	1	1	0
13.	Mr. Tul Bahadur khatri	Peon	None	1	1	0
Total				**13**	**11**	**2**

2.3 Details of budget sanction and expenditure (2062/063)

Budget Line	Budget Heads	Approved Budget (NRs in 000)	Expenditures (NRs in 000)
1.01	Salary	1134	1045
1.03	Transfer TADA	4	3.84
1.4	Dress	11	10.71
1.5	Feed	10	6.98
2.01	Water and Electricity	100	85.67
2.02	Telephone	50	47.02
2.03	Office materials	391	390.84
2.5	Repairs	113	112.3
2.6	Fuel for other purpose	72	70.68
2.7	Service cost	8	7.80
2.8	Miscellaneous	23	22.99
4.02	Medicine	60	59.80
4.04	Program cost	30	30
4.05	Program TADA	149	148.97
6.01	Furniture	15	13.02
6.03	Equipment	40	39.00
Total		**2210**	**2094.62**

3. Laboratory Services

3.1 Diognostic Services

3.1.1 Parasitology

Faecal samples were examined adopting both qualitative and quantitative methods. In the F/Y 2062/63 altogether 974 faecal samples from different species of animals such as cattle, buffalo, sheep, goats, and dogs were examined. Out of 974 faecal samples examined, 356 samples were found to be positive for various internal parasites and 273 samples were negative for any parasites.. Total number of fecal samples of goats examined for EPG count was fifty-seven. Approximately 6000 EPG of GI nematodes were found in the goats of Syangja and Tanahu districts. Fasciola, Paraamphistomum, Coccidia, Strongyloides, strongyls, Trichuris and Monezia were the major internal parasites identified. The results of monthly examinations of faecal samples are presented in Table 2.

Table 2 : Monthly faecal examination results (2062/63)

Parasites	Months (as per Nepalese fiscal year)												Total	+ ve (%)
	4	5	6	7	8	9	10	11	12	1	2	3		
Fasciola	2	5	4	3	2	0	6	4	0	3	0	2	31	8.8
Paramphistome	4	3	4	2	5	3	2	2	1	3	4	6	39	10.9
Ascaris	0	2	2	0	0	0	0	3	0	0	0	0	7	1.9

Strongyle	5	3	4	13	2	0	0	0	7	0	0	22	56	15.7
Strongyloides	3	0	2	0	3	1	0	0	0	0	3	4	16	4.5
Trichuris	0	0	0	0	0	2	0	0	0	0	0	2	4	1.1
Moneizia	0	0	1	2	0	1	0	0	0	0	0	0	4	1.1
Coccidia	7	9	8	12	22	33	12	18	18	3	18	8	168	47.2
Mixed infections	3	0	1	4	2	0	7	0	1	3	0	1	22	6.3
Others (B.coli)	0	1	2	1	0	3	0	0	0	2	0	0	9	2.5
Total positive	**24**	**23**	**28**	**37**	**36**	**43**	**27**	**27**	**27**	**14**	**25**	**45**	**356**	**100**

Twenty-five skin scrapings from goats, cattle and dogs were received for examination and identification of mites. The nine positive samples revealed seven cases of *Sarcoptes* and two cases of *Demodex* spp of mites in cattle and goat, and dogs respectively.

3.1.2 Microbiology

Microbiological examinations include the isolation and identification of bacteria and fungi from the pathological samples received in the laboratory. Bacteriological culture and antibiotic sensitivity tests were performed with the samples received for microbiological investigation. During 2062/63, a total of 552 samples were examined in microbiology unit of the laboratory. Two hundred and eighty-four pathological samples from poultry and other species of animals were subjected for the bacteriological examinations. Similarly 268 milk samples were tested for mastitis using Sodium Lauryl Sulphate test (SLST) among which 147 milk samples were screened as positive for SLST. The SLST positive milk samples were subjected for bacteriological culture and the isolates were subjected for antibiotic sensitivity test. Out of 147 SLST positive milk samples, 119 samples resulted in bacterial growth results of which are presented in Table 3.

Results of antibiotic sensitivity test revealed that Enrofloxacin followed by Cefotaxime and Gentamicin were found to be the most sensitive antibiotics against the bacteria isolated from milk of cattle and buffaloes affected with mastitis. It may therefore, be concluded that these antibiotics could be used for the routine treatment of mastitis cases in the district where culture and antibiotic sensitivity testing of milk samples are not possible. Results of antibiotic sensitivity testing of bacterial isolates are given in Table 4.

In chronic cases of mastitis, milk samples were also cultured in Saboraud's Dextrose Agar for the fungus culture and identification. Seven milk samples were found to be positive comprising five case of *Candida* spp and two cases of *Absidia* spp of fungus.

Table 3 : Bacterial isolates from different types of samples

Bacterial Species	Number of isolates from milk samples	Number of isolates from other samples
Staphylococcus	38	24
Streptococcus	17	9
Bacillus spp.	28	23
Pasturella multocida		2
Proteus	9	7
Micrococcus	3	3

Corynaebacterium	5	3
Enterobacter	4	4
Salmonella		2
Escherichia coli	54	37
Pseudomonas	5	3

Table 4 : Antibiotic sensitivity of the isolates of milksamples

Antibiotics used	Percent sensitivity
Enrofloxacin	99.38
Cefotaxime	96.93
Gentamicin	88.91
Chloramphenicol	87.34
Kanamycin	86.88
Oxytetracycline	34.23
Amoxycillin	38.12

3.1.3 Pathology

Pathological examinations mostly consist of post mortem examination (PM) of animals and poultry. Seven hundred seventy one poultry, 7 piglets and 2 were brought for the postmortem examination. Three piglets were found to have died due to pasturellosis and 4 died due to enteritis. Two goats were found to have died due to G.I. nematodiasis. In the pathology unit, the cause of death of chickens presented was generally drawn on the basis of both the post mortem lesions observed and laboratory investigation of samples collected during PM examinations. Some times samples collected during PM were sent to Central Veterinary Laboratory, Tripureswor for the confirmatory diagnosis. Diseases of chickens diagnosed are summarized in Table 5.

Table 5 : Trend of disease occurrence in poultry

S. N.	Disease diagnosed	Total cases	Prevalence (%)
1	Infectious Bursal disease	178	22.25
2	Haemorrhagic enteritis	88	11.12
3	Coccidiosis	82	10.36
4	Colibacillosis	68	8.56
5	Chronic respiratory disease	58	7.33
6	Miscellaneous disease	39	4.93
7	Visceral gout	36	4.55
8	Leechi Heart disease	34	4.29
9	Salmonellosis	33	4.17
10	Mycotoxicosis	32	4.04
11	Ascitis	29	3.66
12	Omphalitis	28	3.53
13	Hepatitis	18	2.27

14	Vitamin/Mineral deficiency	14	1.76
15	Ranikhet disease	11	1.39
16	Ascariasis	8	1.01
17	Fowl cholera	5	0.63
18	Fowl pox	4	0.05
19	Leucosis	4	0.05
20	Marek's disease	4	0.50
Total		**771**	**100**

3.1.4 Serology

Serological examinations mainly includes plate agglutination test of chicken serum to detect antibody against *Mycoplasma gallisepticum, Mycoplasma synoviae* and *Salmonella pullorum*. Agar gel precipitation test was used for the detection of antibody in chicken against avian encephalomyelitis virus. Similarly, serum samples from cattle, buffalo, sheep and goats were tested for brucella antibodies using Rose Bengal plate agglutination test. During the fiscal year 2062/63, the serum samples tested and their results are presented in table 6.

Table 6 : Results of serum samples tested

Species	Number of serum tested	Test request	Test applied	Result
Cow	32	Brucellosis	RBPT	1 positive
Buffalo	18	Brucellosis	RBPT	1 positive
Goat	26	Brucellosis	RBPT	All negative
Poultry	226	Mycoplasmosis	PAT	26 positive
Poultry	226	Salmonellosis	PAT	22 positive
Pig	7	Swine fever	AGPT	All negative

3.1.5 Haematology

Hematological unit of the laboratory is well equipped to determine a range of hematological parameters such as total erythrocyte count and total leukocyte count, differential leucocytes counts, erythrocyte sedimentation rate (ESR), determination of hemoglobin content, and packed cell volume and staining of blood smears for the evaluation of the presence of blood protozoa and bacteria in blood. A total of 304 blood samples from animals were examined for different hematological parameters among which 85 blood samples were examined for the presence of blood parasites which revealed *Babesia* spp in 3 cows, *Anaplasma* spp. in 3 cows and *Dirofillaria immitis* in a dog.

3.1.6 Biochemistry

Biochemical examinations included biochemical analysis of serum and routine and microscopic examination of urine. Multistick strip was used for routine urine analysis. Microscopic examination of urine was done after centrifugation of the urine samples. Similarly biochemical parameters of serum samples were determined using spectrophotometer and commercially available biochemical kits. A total of 226 samples were examined in biochemistry unit including

109 urine samples. Out of 109 urine samples, 63 cases were diagnosed as haematuria, 12 cases as proteinuria and 32 included miscellaneous conditions.

3.2 Other Services

3.2.1 Sample collection and dispatch

During 2062/63, a total of 703 serum, blood and tissue samples of different species of animals and poultry were collected from the disease outbreak investigation sites. Among them, 502 samples were subjected for the laboratory investigation at RVL, Pokhara. A total of 184 samples were dispatched to central veterinary laboratory and 17 samples to national FMD and TADs laboratory for confirmative diagnosis. Details of samples referred to different laboratories are presented in table 8.

Table 8 : Sample referred to different reference laboratories

S. N.	Species	District	Type of samples	Total samples	Test request	Ref. lab	Test method
1	Goat	Kaski	Serum	15	PPR	CVL	C-Elisa
2	Goat	Myagdi	Serum	5	PPR	CVL	C-Elisa
3	Goat	Arghakhanchi	Serum	20	PPR	CVL	C-Elisa
4	Goat	Rupendehi	Serum	50	PPR	CVL	C-Elisa
5	Goat	Gulmi	Serum	47	PPR	CVL	C-Elisa
6	Pig	Kaski	Serum	5	Swine fever	CVL	AGPT
7	Goat	Baglung	Serum	2	PPR	CVL	C-Elisa
8	Goat	Kaski	Serum	5	PPR	CVL	C-Elisa
9	Goat	Myagdi	Serum	8	PPR	CVL	C-Elisa
10	Poultry	Kaski	Spleen, liver, lungs	3	ND	CVL	Virus isolation
11	Bovine	Gorjha, Nawalparasi, Gulmi, Shyanja	Serum	36	Biochemical estimation	CVL	Com. kits
12	Goat	Kaski	Mouth lesion scrapping	1	FMD	FMD & TADs lab	ELISA
13	Buffalo	Baglung	"	5	FMD	"	ELISA
14	Cow, Goat	Syangja	Mouth lesion scrapping	3	FMD	FMD & TADs lab	ELISA

3.2.2 Disease Investigation

3.2.2.1 Investigation of epidemics

Various disease outbreaks of animal and poultry were investigated during fiscal year 2062/63. Whenever request for investigation of an outbreak was received from the district to the RVL, a veterinarian or a technician or a team of technicians with necessary sampling kit visited to the site of epidemic, collected epidemiological information and appropriate pathological samples. In the laboratory, pathological samples collected from the field were processed to find out the etiology of the outbreak. Epidemiological information gathered from the site of an outbreak was used to decide the test to be performed in the laboratory and to assist in the confirmation of disease diagnosis. Samples, not possible to process in this laboratory were referred to CVL, Kathmandu.

A total of 10 epidemics were investigated during 2062/63. Of 10 outbreaks investigated, some were confirmed by laboratory while others confirmations were based on clinical signs and postmortem findings. The epidemiological findings of the outbreaks are presented in Table 9.

Table 9 : Result of outbreaks investigation (2062/63)

S.N.	Date of outbreak	District	Animal spp affected	Disease diagnosed	Animal at risk	No. of animal affected	No. of animal died
1	Shrawan 2062	Syangja	Poultry	Ranikhet	460	460	296
2	Bhadra 2062	Tanahu	Goat	G.I. nematodes	240	65	8
3	Asoj 2062	Syangja	Cattle	FMD	211	8	0
4	Ashoj 2062	Kaski	Buffalo	H.S.	132	4	2
5	Kartik 2062	Kaski	Mule	Candidiasis	23	5	2
6	Kartik 2062	Syangja	Goat	GI Nematodes	262	25	9
7	Mangshir 2062	Kaski	Pig	H.S.	35	9	4
8	Falgun 2062	Kaski	Pig	H.S.	16	4	4
9	Jestha 2063	Baglung	Goat	PPR	344	9	5
10	Asadh 2063	Kaski	Poultry	Ranikhet	850	600	300

3.2.2.2 Investigation of Mycoplasmosis in livestock

A total of 40 nasal swabs of goats suffering from respiratory disorders from Myagdi, Kaski, Tanahu and Syangja were collected and subjected for Mycoplasma isolation. Similarly 7 pneumonic lungs from dead goats were collected and subjected for Mycoplasma isolation. No mycoplasma could be isolated.

3.2.2.3 Mycobacterial disease investigation

Single Intradermal Tuberculin test of 11 bulls and six buffalo bulls of National Animal Breeding Centre, Pokhara were carried out. These test revealed all negative results for bovine tuberculosis.

3.2.3 Surveillance Programme

3.2.3.1 Bird flu

For Sero-surveillance of bird flu a total of 75 serum samples were collected from commercial and village poultry. Similarly 62 Cloacal swabs were also collected. The serum and Cloacal swabs were sent to the Central Veterinary Laboratory (CVL) Kathmandu for laboratory investigation of bird flu. The laboratory diagnosis result obtained from CVL, Kathmandu revealed all the samples negative for bird flu.

3.2.3.2 PPR vaccination programme

During the fiscal year 2062/63 Sero-surveillance of PPR vaccination in the sheep and goats programme in the western region was carried out. A total of 720 serum samples from Tanahu, Shyanja, Parvat, Myagdi, Baglung, Kaski, Nawalparasi, Rupendehi and Kapilvastu were collected from the vaccinated sheep and goats. All the collected serum samples were dispatched to Central Veterinary Laboratory for the examination of antibody against PPR vaccination.

Regional Veterinary Laboratory
(Mid-Western Region)

1. Introduction

The Regional Veterinary Laboratory of mid-western region is located in Birendranagar municipality of Surkhet district was established in F/Y 1988/1989 AD. This laboratory provides diagnostic services in all the fifteen districts of Bheri, Rapti and Karnali zone of Nepal. The diagnostic activity was formally initiated from F/Y 1991/1992 when workforce, essential equipments and other logistic supports were provided through various organizations. The most remarkable step was achieved during 1997-1998 with the technical support provided by **Strengthening of veterinary services for livestock disease control** project.

As do other diagnostic laboratories, RVL, Surkhet also receives various specimens from different sources. Majority of the specimens are received from different DLSOs and individual farmers. However, a substantial number of specimens are also collected by the laboratory itself during surveillance and epidemic investigations.

2. Programme and progress

Annual programme and progress, RVL, Surkhet (2062/063)

S. N.	Programme	Unit	Annual target	Annual progress	Progress %
1	**Laboratory services**				
1.1	Parasitological examination	Number	1000	1218	100 %
1.2	Microbiological examination	Number	300	405	100 %
1.3	Pathological examination	Number	300	300	100 %
1.4	Serological examination	Number	400	849	100 %
1.5	Haematological examination	Number	300	300	100 %
1.6	Biochemical examination	Number	200	362	100 %
1.7	Sample to be sent to other lab.	Number	350	1873	100 %
2	**Investigation & surveillance programme**				
2.1	Investigation of respiratory problems in goat	Times	12	12	100 %
2.2	Investigation of epidemic disease	Times	6	6	100 %
3	**Supervision & monitoring programme**				
3.1	Supervision & monitoring of district laboratories	Times	12	12	100 %
4	**Veterinary disease investigation workshop**	**Times**	**1**	**1**	**100%**
5	**Training programme**				

5.1	Laboratory training for JT/JTAs.	Members	2	2	100%
6	**Publication programme**				
6.1	Publication of quaternary epidemiological bulletin	Times	4	4	100 %
6.2	Publication of annual technical report	Times	1	1	100 %
7	**Purchase**				
7.1	Technical books	Times	1	1	100 %

3. Laboratory services

3.1 Diagnostic Services

Laboratory services include examination of samples of faeces, skin scrapings, urine, milk, blood etc. Various diagnostic techniques are conducted through different laboratory units, the function and progress have been present below.

3.1.1 Parasitologiy

Parasitology laboratory unit involves the examination of faeces and skin scrapings. Generally, faecal samples are studied qualitatively but quantitative examination of the faecal sample is also done. Altogether, 1218 faecal samples from different species of animals; cattle, buffalo, goat and dog were received during 2062/063. Among them, nine hundred and seventy samples (79.63%) were found positive whereas 253 samples (20.77%) were negative. The result of the faecal test revealed that fascioliasis was the most commonly occuring parasitic infestation in buffaloes and cattle followed by nematodiasis and paramphistomiasis. A detail about the faecal examination has been presented in table 1.

Table 1 : Monthly faecal examination result (2062/063)

Months	Sample		Fasciola	Par	Tri	Haemonchus	Ascarids	Others	Total
	+ ve	- ve							
July	80	30	52	12	-	6	10	7	110
August	69	21	42	8	5	2	-	6	90
Sept.	50	10	28	9	2	3	-	7	60
Oct.	54	16	42	9	8	5	2	6	70
Nov.	92	27	52	15	6	4	-	5	119
Dec.	99	31	65	14	7	5	-	4	130
Jan.	85	20	52	12	7	2	6	3	105
Feb.	75	15	42	8	3	5	-	2	90
March	75	21	70	13	6	3	-	5	96
April	122	23	82	13	2	4	7	3	145
May	85	16	61	14	9	3	-	3	101
June	84	18	56	11	5	4	-	4	102
Total	**970**	**253**	**646**	**138**	**60**	**46**	**25**	**55**	**1218**
Percent	**79.63%**	**20.77%**	**66.59%**	**14.22**	**19.17%**				

(Note : 'Par' stands for Paramphistomum spp and 'Tri' stands for Trichuris spp.)

Skin scrapings are collected from animals suffering from skin lesions and examined for identification of mites. Altogether 33 samples from different species of animals were received and examined which revealed the presence of *Sarcoptes* spp of mites in most cases.

3.1.2 Haematology

Haematology laboratory unit deals with all the haematological examinations; total leucocytic count (TLC), total erythrocytic count (TEC), differential leucocytic count (DLC), estimation of packed cell volume (PCV) and haemoglobin (Hb). A total number of 300 blood samples were examined during the fiscal year 2062/063.

3.1.3 Microbiology

Milk samples from the suspected cases of mastitis constitute the majority of microbiological samples. However, other types of samples are also collected from post-mortem laboratory unit and field condition The milk samples screened as positive for the occurrence of mastitis through California mastitis test (CMT) are put for bacterial culture and the isolates are subjected to antibiotic sensitivity test to study the efficacy of commonly used antibiotics against various bacteria.

Altogether 175 milk samples were received in microbiology laboratory unit during 2062/063. The most common bacterial isolates were *Streptococcus, Staphylococcus, and E.coli.* The result of CMT test has been presented in table 2. Similarly, the efficacy of various commonly used antibiotics has been presented in figure 1 below.

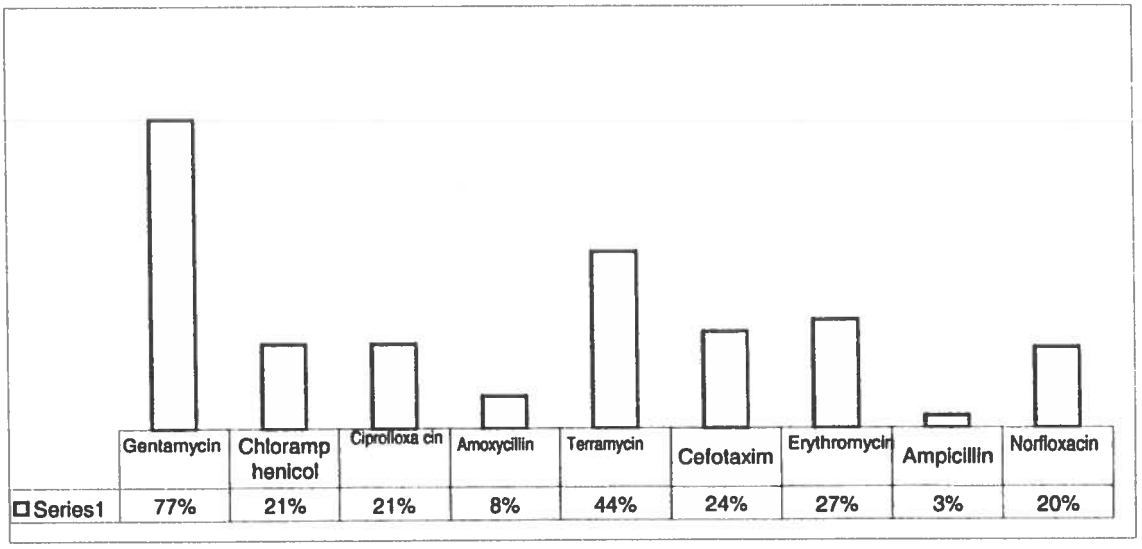
Table 2 : Result of CMT test

Months	Total sample	Positive	Negative	% Positive
July	32	16	16	50.00
August	18	10	8	55.55
Sept.	9	5	4	55.55
Oct.	6	3	3	50.00
Nov.	8	5	3	62.50
Dec.	8	4	4	50.00
Jan.	7	3	4	42.85
Feb.	11	5	6	45.45
March	12	7	5	58.33
April	14	8	6	57.14
May	22	11	11	50.00
June	28	15	13	53.57
Total	**175**	**92**	**83**	**52.57**

3.1.4 Biochemistry

This unit involves the routine examination of urine and analysis of blood serum to analyze the various biochemical constituents. Analysis of blood serum is done for the estimation of total protein, glucose, phosphorus using commercial kits. Urine samples are tested by using multisticks as well as traditional biochemical methods. Examination of urine is done for specific gravity and pH, and to explore the presence of sugar, albumin, ketone bodies, urobilinogen and blood.

Figure 1 : Result of antibiotic sensitivity test

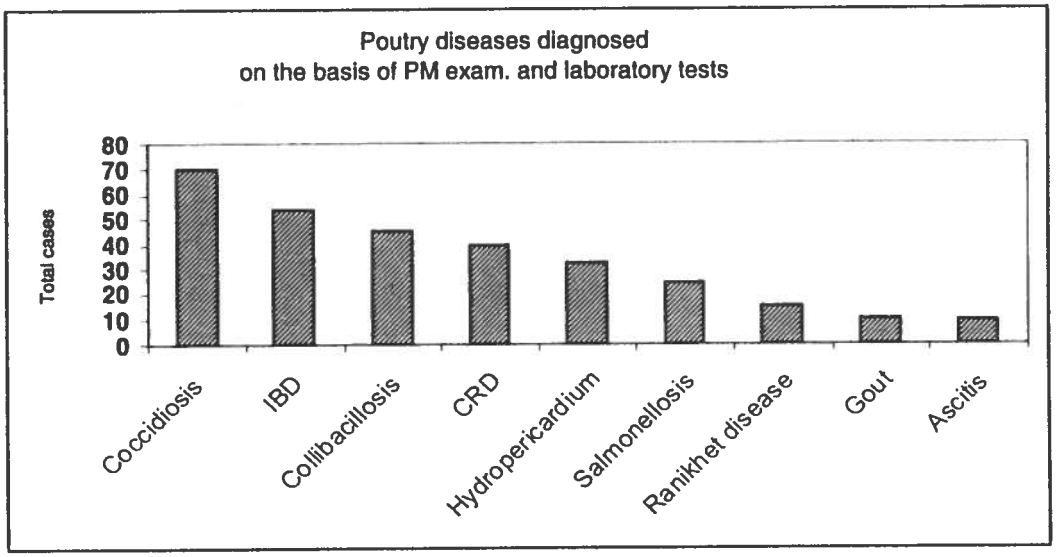


3.1.5 Pathology

Mostly post-mortem examinations of dead birds and occasionally of dead animals are done in the laboratory or in field condition.. During post-mortem examination, specimens such as impression smears, swab, and tissues are collected and tested accordingly. Specimens intended for microscopic pathology are sent to the Central Veterinary Laboratory as this facility is not available in this unit.

Among the 300 cases of poultry birds received for PM examination, Coccidiosis was found to be the most common disease followed by IBD, Collibacillosis, CRD, Hydropericardium and Ranikhet disease. The status of various diseases diagnosed during 2062/063 has been presented in figure 2.

Figure 2 : Status of various diseases/conditions in commercial poultry (2062/063)



3.2 Other Services

3.2.1 National PPR programme

The vaccination and serum collection during F/Y 2062/063 from different districts has been presented in table 3 below.

S. N.	DLSOs	Total vaccination target	Total serum collection	Remarks
1	Dailekh	50	50	
2	Surkhet	50	50	
3	Banke	75	75	
4	Bardia	75	75	
5	Jumla	25	25	
6	Humla	25	25	
7	Rolpa	50	50	
8	Salyan	50	50	
9	Pyuthan	50	50	
10	Dang	100	100	
11	Jajarkot	25		Vaccination not done
12	Rukum	25		Collection couldn't be done
Total		**600**	**550**	

Regional Veterinary Laboratory
(Far-Western Region)

1. Introduction

Regional veterinary laboratory of far western development region is located in Dhangadhi municipality of kailali district. It provides diagnostic services to all the nine districts of the region. These districts are distributed in two zones; Seti and Mahakali. It is the smallest region among the five development regions. Geographically the region is divided into three parts namely; mountains, hills and terai. RVL, Dhangadhi performs various diagnostic activites through its several laboratory units; Pathology, Parasitology, Microbiology, Biochemistry, Haematology and Serology. Besides, washing and sterilization, and epidemiology unit serve therir respective activities. The various sources of specimens are DLSOs, farmers and field where laboratory technicians collect specimen during surveillance programme and epidemic investigations.

2. Programme and Progress

2.1 Annual program and progress of RVL, Dhangadhi (2062/63)

S.N.	Programs and Activities	Unit	Annual Target		Annual Progress	Percent Progress
			Quantity	Weightage		
1	**Laboratory Services**					
1.1	Parasitological Examination	Number	1000	3.51	2579	100
1.2	Microbiological Examination	Number	300	4.51	343	100
1.3	Pathological Examination	Number	400	4.01	332	83
1.4	Serological Examination	Number	350	3.51	371	100
1.5	Hematological Examination	Number	300	3.01	350	100
1.6	Biochemical Examination	Number	400	6.01	369	92.25
1.7	Dispatch of samples (CVL and other Lab	Number	250	2.51	434	100
2	**Disease Investigation and Surveillance Programme**					
2.1	Investigation and surveillance of kid mortality	Time	12	11	12	100
2.2	Investigation and surveillance of Khari Disease.	Time	12	34.1	12	100
2.3	Investigation of Epidemic Diseases	Time	6	7.52	6	100

3	**Inspection and Supervision Programme**					
3.1	Inspection and Supervision of District Labs.	Time	6	5.41	6	100
4	**Annual workshop on Animal Disease Investigation)**	**Time**	**1**	**2.51**	**1**	**100**
5	**Training Programme**					
5.1	Refresher laboratory training for JT/JTA	Person	7	6.51	7	100
6	**Publication**					
6.1	Quarterly Epidemiological bulletin publication	Time	4	2.4	3	75
6.2	Annual Technical Book Publication	Time	1	0.50	1	100
7	**Purchase**					
7.1	Purchase of scientific Books and Journals	Time	1	1	1	100

Note : Animal Health Services Programme : 97.07%, Weightage of progress : 93%.

2.2 Details of budget expenditure, RVL, Dhangadhi (2062/063)

Budget line	Budget head	Approved budget (Rs)	Received budget (Rs)	Expenditure (Rs.)
1.01	Salary	1145000.00	1143179.54	1143179.54
1.02	Allowance			
1.03	TADA &Transfer Expense	21000.00	3998.00	3998.00
1.04	Dress	12000.00	11750.00	11750.00
1.05	Food and Nutrition	8000	7692	7692
2.01	Water and Electricity	105000.00	102162.35	102162.35
2.02	Telephone	66000.00	62232.31	62232.31
2.03	Office concerned	417,000.00	411396.00	411396.00
2.05	Repairs	94,000.00	92097.00	92097.00
2.06	Fuel for vehicle & other use	91,000.00	90831.00	90831.00
2.07	Consultancy & other services	10,000.00	9640.00	9640.00
2.08	Miscellaneous	25,000.00	24400.00	24400.00
4.02	Medicine purchase	60,000.00	60,000.00	60,000.00
4.04	Programme Expense	110,000.00	109909.00	109909.00
4.05	Programme TA/DA	160,000.0	159917.00	159917.00
6.01	Furniture	20,000	20,000	20,000
	Total	**23,44,000**	**2289503.50**	**2289503.50**

2.3 Description of human resource, RVL, Dhangadhi (2062/063)

S. N.	Name of the staff	Post	Class	Sanctioned	Available	Vacant
1.	Dr. Bimal Kumar Nirmal	Senior VO	GII	1	1	-
2.	Dr. Raju Gautam	VO	GIII	1	1	-
3.	Mr. Shyam Prasad Pathak	JT	NGI	1	1	-
4.	Mr.Anil Man Sob	JTA	NGII	1	1	-
5.	Mr. Hari Singh Bhandari	JTA	NGII	1	1	-
6.	Ms.Menaka Shrestha	Typist	NGI	1	1	-
7.	Mr. Jai Bdr. Pal	Store keeper	NGII	1	1	-
8.	Mr. Ganesh Boahra	Accountant	NGII	1	1	-
9.	Mr. Keshav Raj Pandey	S. man	NGIII	2	1	1
10.	Mr. Shankar Prasad Paudel	Driver	None	1	1	-
11.	Mr. Tilak Bdr. Malla	Peon	None	1	1	-
12.	Prem Bahadur Chaudhary	Peon	None	1	1	-
	Total			**13**	**12**	**1**

3. Laboratory Services

3.1 Diagnostic Services

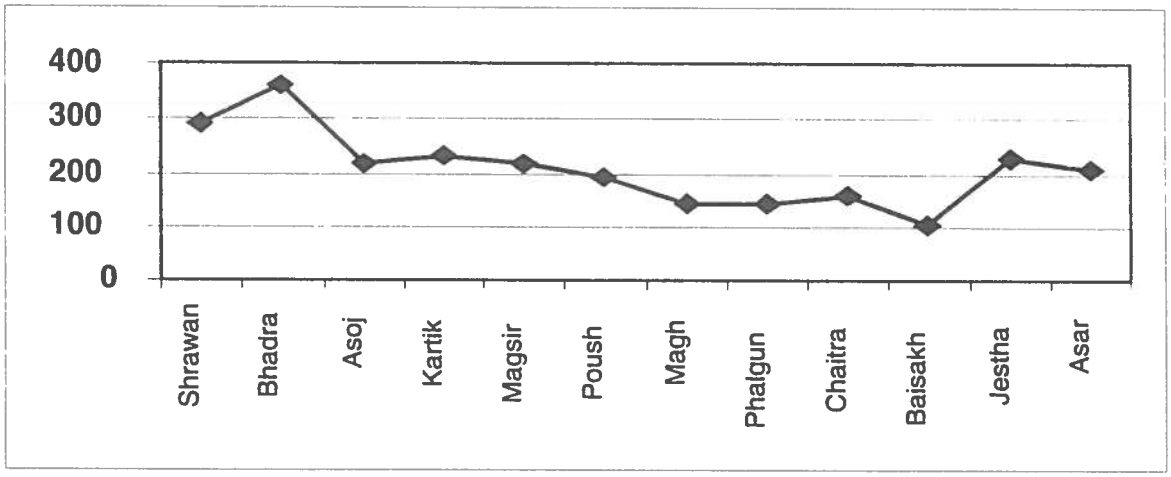
3.1.1 Parasitology

Altogether two thousand and six hundred sixteen samples form animals and poultry were examined at parasitology laboratory unit. Among them, 2225 (85.05%) were positive and only 391 (14.95%) were negative. It was found that most of the faecal samples examined were having mixed parasitic population. The most common helminths identified during faecal examination was *Fasciola* spp followed nematodes namely, *Strongylus* spp, Stronglyloids, Ascarids, *Trichuris* spp, Coccidian parasites, and the cestodes; the *Moniezia* spp. Table 1 shows monthly prevalence of different parasites. The prevalence of internal parasites in livestock population is presented in figure 1. Later suggests that cases of parasitic infestations increase from Jestha onwards reaching the highest cases of infestation during Bhadra and moderate and uniform infestations have been seen from Asoj to Baisakh. A parasitic infestation has been observed significantly higher during summer and rainy seasons, which drops with the onset of autumn and remains relatively constant until the end of spring season.

Table 1 : Monthly prevalence of different internal parasites

Parasites (Spp)	4	5	6	7	8	9	10	11	12	1	2	3
Fasciola	181	122	101	84	54	58	56	18	60	28	110	47
Paramphistomes	29	145	77	75	67	39	44	92	67	47	57	108
Strongylus	31	42	24	34	42	39	18	18	10	8	19	13
Strongyloides	19	15	6	17	15	19	3	4	4	2	14	3
Ascarids	6	7	4	3	4	6	7	0	7	3	21	11
Coccidia	5	11	1	2	15	10	9	2	7	8	0	1
Others	18	20	5	16	20	19	13	2	5	7	7	22
Total	**289**	**362**	**218**	**234**	**217**	**191**	**144**	**136**	**160**	**103**	**228**	**205**

Figure 1 : Monthly examination of faecal samples (2062/063)



3.1.2 Serology

A total of 371 serological tests of different types were performed against the target of 350 during the fiscal year 2062/63. Most of the serum samples collected were from goats for various diagnostic tests viz. PPR, Mycoplasma, Brucellosis and other disease conditions responsible for causing abortion in these animals, the samples so obtained were forwarded to CVL. The detail of serological tests performed on serum of various animals is presented in the table below along with the results. Poultry serum was mainly used as diagnostic aid of two major disease conditions namely Salmonella and Mycoplasma.

Serum samples collected from bovine population were mainly under the Khari disease investigation program. These samples were obtained from Baitadi and Darchula and forwarded to CVL for necessary biochemical test of the serum apart from performing the regular screening test of Brucellosis using Rose Bengal Plate Agglutination Test. The sera of humans and pigs were collected for investigation of Japanese Encephalitis (J.E.) program running under CVL. Human serums were collected from suspected cases of J.E. brought to Seti Zonal Hospital, Dhangadhi. The results of serological tests conducted at RVL, Dhangadhi and the results of the same received from CVL have been shown in table 2 and 3 respectively.

Table 2 : Result of Serological tests conducted at the RVL, Dhangadhi (2062/063)

S. N.	Spp of animal	Total sample	Diseases								Referred to CVL
			Salmonella		Mycoplasma		Brucellosis		Tuberculosis		
			+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
1	Bovine	62					0	33			62
2	Sheep/goat	172					0	87			172
3	Poultry	81	7	56	9	76					5
4	Humans	12									12
5	Pigs	20									20

Table 3 : Result of Serological tests received from CVL

S. N.	District	Animal spp.	No. of sample	Date	Test requested	Result	
						+ve	-ve
1	Kanchanpur, AQO	Goat	22	2062/5/4	PPR	8	14
2	Kailali, Godavari	Goat	11	2062/5/27	PPR	1	10
3	Banbedha, Kailali	Goat	10	063/3/8	PPR	10	0
4	Chaumala, Kailali	Goat	2		PPR	2	0
5	Mahendranagar, Kanchanpur	Goat	10		PPR	10	0
6	Jhalari, Kanchanpur	Goat	6		PPR	3	3
7	Darchula	Goat	17	2063/3/9	PPR	14	3
8	Kanchanpur	Poultry	5		Bird flu	0	5

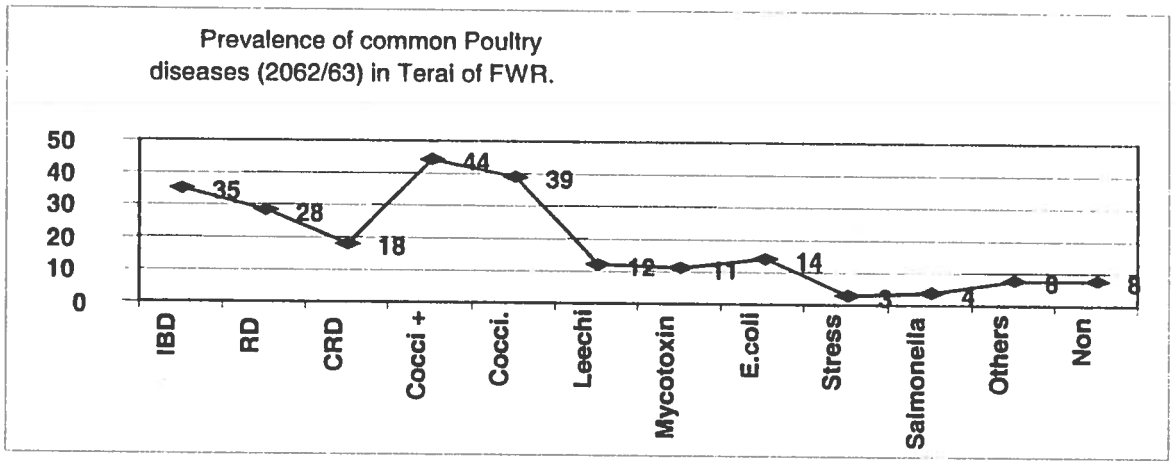
3.1.3 Haematology

Haematological examination includes TLC, DLC, TEC, PCV, Hb, ESR and examination of blood smears for presence of blood parasites. A total of 163 blood samples were collected during epidemic outbreaks and from animals selected for the specific investigation programme of the laboratory. Rest of the cases was received from DLSO Kailali.. Among 163 blood smears examined, only two of the samples were found positive for *Babesia* spp. Similarly, examination of blood samples collected from buffaloes under the Khari disease investigation programme invariably showed low Hb concentration ranged from 5.4 - 7.6 g/dl.

3.1.4 Pathology

Gross examination of the dead animals and birds are conducted at pathology laboratory unit. During the F/Y 2062/063, three hundred and thirty-four postmortem cases were received which included six cases of goats and two cases of wild pigs received from Suklaphanta wild life reserve, Kanchanpur, and rest of the cases of poultry. The cases of goats were diagnosed as pneumonia, jaundice, kumri and parasitic infestations and that for pig were haemorrhagic septicemia and parasitic infestation with Macrocanthorhincus hirudinaceus Figure 2 reveals the picture of various diseases/conditions diagnosed in poultry at RVL, Dhangadhi Coccidiosis (+) as mentioned in the figure indicates conccurrence with other common diseases. Besides, one brain sample collected from a yearling buffalo she calf was confirmed as the case of Rabies from CVL.

Figure 2 : Common poultry diseases of Far Western region



3.1.5 Microbiology

The samples subjected to microbiological examination constitute of milk, nasal swab, vaginal swab, and swab from visceral organs like liver, lungs, intestine etc. of various animal species. The milk samples screened as positive for California mastitis test were subjected to bacterial culture and isolation. The various isolates were challenged with various commonly used antibiotics to study the efficacy of the latters. The result of antibiotic sentivity test is presented in table 4.

Table 4 : Result of Antibiotic Sensitivity test for Milk Samples

Antibiotic	Total no. of tests	Sensitive (%)	Resistant (%)	Intermediate (%)
Gentamycin	46	34 (74)	8 (17)	4 (9)
Ceprofloxacin	42	30 (71.4)	4 (9.52)	8 (19)
Tetracyclin	46	6 (13)	38 (82.6)	2 (4)
Cloxacillin	46	2 (4.3)	44 (95.6)	0
Ampicillin	46	2 (4.3)	44 (95.6)	0

3.1.6 Biochemistry

Biochemistry laboratory unit received urine and serum samples for biochemical evaluation. Altogether thirty-nine urine samples were examined using multisticks for detection of urobilinogen, protein, pH, blood, specific gravity, ketone bodies, glucose and bilirubin. Among them, 18 urine samples were found to be positive for the presence of ketone bodies and 21 samples were negative for the occurrence any commonly found abnormality.

3.2 Other Services

3.2.1 Epidemic investigation

The number and the epidemic investigation carried out during F/Y 2062/063 is given in the table 5.

Table 5 : Disease outbreak in the far western region during the fiscal year 2062/063

Month	Year	District	VDC	Species	Disease
Bhadra	2062	Dadeldhura	Jogbuda	Cattle	Ergot poisoning
Mangsir	2062	Kailali	Chaumala	Goat	Kumri
falgun	2062	Kailali	Godavari	Goat	PPR
Chaitra	2062	Kailali	Sahajpur	Goat	Babesiosis
Baisakh	2063	Kailali	Ghorsuwa	Goat	PPR
Jestha	2063	Dadeldhura	Jogbuda	Goat	PPR
Asar	2063	Darchula		Goat	PPR

3.2.2 Bird Flu surveillance

Bird flu surveillance was carried out both passive and active. Under passive surveillance over 46 commercial farms were inspected in different districts and observation of Suklaphanta wild life reserve in Kanchapur district along with important natural lakes; Ghodaghodi located in Sandepani village development committee of Kailali district. Similarly, active surveillance was conducted and alogether 115 cloacal and tracheal swabs, 15 poultry serum, and six dead birds including poultry were collected and dispatched to the CVL for laboratory examination.

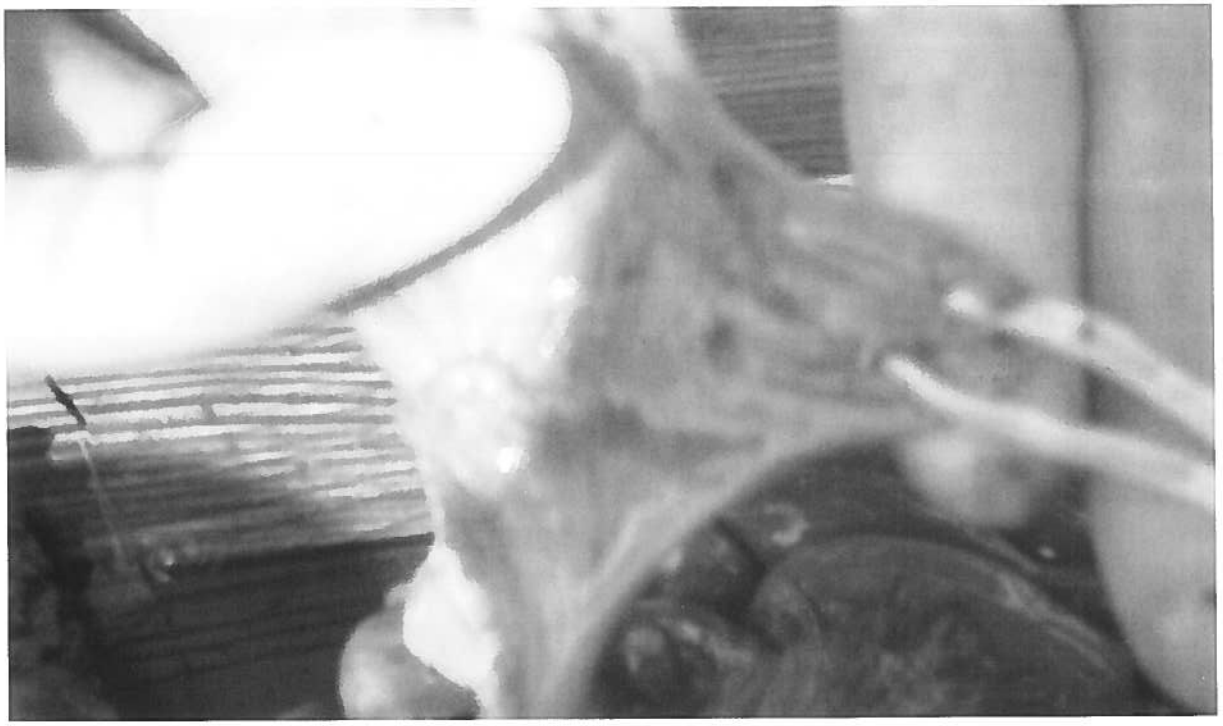


Figure 1 : Macrocanthorhincus hirudinaceus attached to the intestinal wall with typical granulomatous lesion (Curtsey : RVL Dhangadhi)

Figure 2 : Penetration of the intestinal wall with Macrocanthorhincus hirudinaceus causing peritonitis (Curtsey : RVL, Dhangadhi)

Figure 3 : A clinical case of mastitis in buffalo (Curtsey : RVL, Dhangadhi)

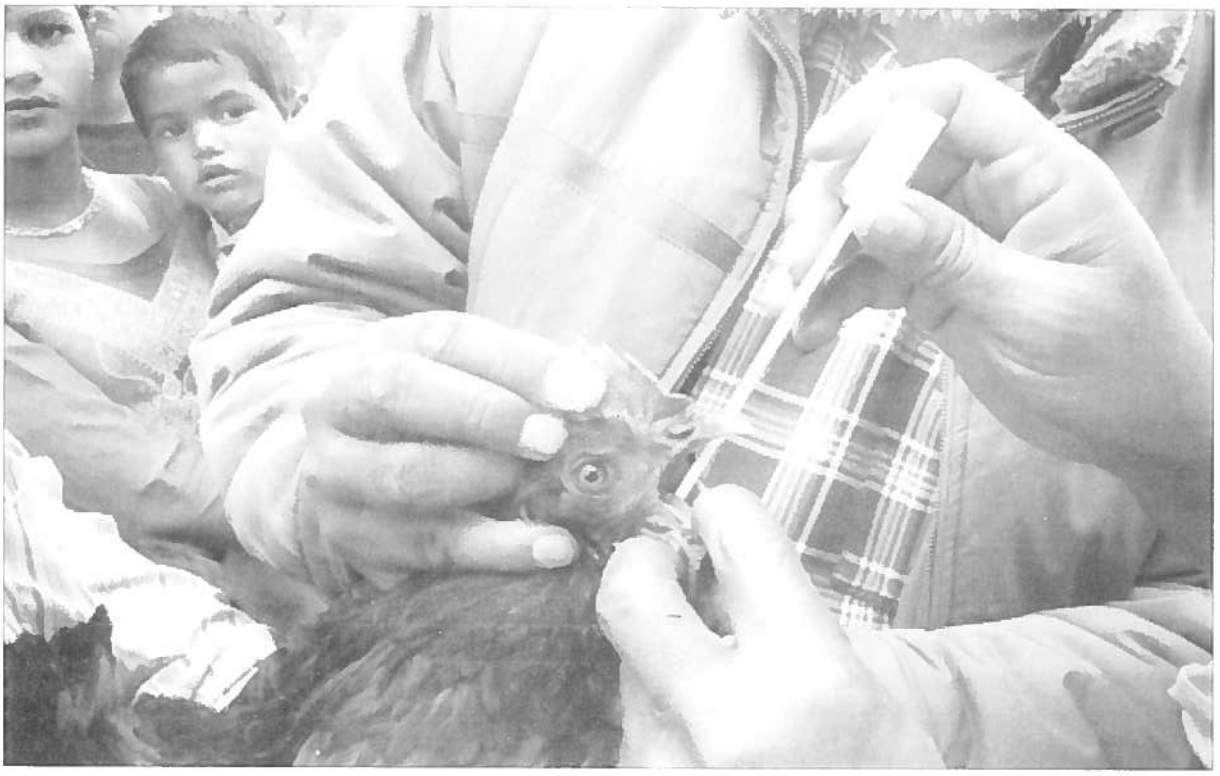


Figure 4 : Technician of RVL, Dhangadhi collecting tracheal swab from a local poultry at Gokuleshwor, Darchula (Curtsey : RVL, Dhangadhi)

Serological study of Japanese Encephalitis virus in pigs, horses and ducks in Nepal

Dr. Ganesh Raj Pant
Senior Veterinary Officer
Central Veterinary laboratory

Abstract

Japanese encephalitis (JE) is an emerging viral disease, which causes encephalitis in human being, horses and abortion in pigs. Pigs and ducks are considered to be the most important amplifying host for JE virus. A serological study for JE was conducted in Nepal from 2002 to 2005. In total, 633 samples were collected randomly from 19 districts; 534 sera of pigs, 25 of horses and 74 of ducks. Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) was used for the detection of antibodies against JE virus. The sero-prevalence of JE in pigs, horses and ducks was 57.67%, 68.0% and 21.62 % respectively.

Key words *: Japanese encephalitis, Emerging disease, and Competitive Enzyme-Linked Immunosorbent Assay, Nepal*

Summary

Sero- prevalence of JE in pigs, horses and ducks was found to be 57.67%, 68.62% and 21.62% respectively.

Introduction

Japanese encephalitis is a mosquito–borne disease of human and animal caused by *Flavivirus*. It is a disease of major public health importance in Nepal. This disease was first reported in Rupandehi district in 1978 (1). Since then, seasonal outbreak of JE has been reported causing around 1000–3000 cases and 200 to 400 deaths annually. The mean clinical attack rate of JE has been reported 1 :300 and neurological sequels have been reported in 30% of recovered patients (2). According to Nepal Health Authority, 30,995 cases and 5,830 deaths were reported from 1978 to 2005. Highest case fatality (46.2 %) was reported in 1982 (3). The prevalence of JE infection has been reported 62% in 1 to 15 years old children (4). Epidemic of JE usually occurs with the onset of monsoon season in July and ends in October. Japanese encephalitis is prevalent in 24 terai and inner terai districts of the country. The population at risk has been estimated about 12.5 million (2). The number of JE cases and death reported in Nepal in 2005 were 2784 and 316 respectively (Personal communication with Epidemiology and Diseases Control Division). The Culex mosquito (Cx. *tritaeniorhyncus*) is considered as a principal vector for the transmission of JE in Nepal (2). The total irrigated land for cultivation of rice is 771,759 hectors in Nepal, which acts as a favorable environment for the breeding of Culex mosquito (5). There are four referral laboratories for the diagnosis of JE in Nepal; National Public Health Laboratory, Kathmandu, Vector Borne Disease Research and Training Center, Hetauda, B.P. Koirala Institute of Medical Science, Dharan, JE Laboratory, Nepalganj. Confirmatory Laboratory diagnosis of human cases is

carried out by IgM C-ELISA. Reconfirmation of JE diagnosis is being conducted at Armed Force Research Institute of Medical Science Thailand. JE virus isolation laboratory facility is not available in Nepal till now. Limited number of children has been immunized annually for the control of the disease due to lack of vaccine. A total number of 481,421 children aged between 6 to 10 years were vaccinated against JE during 2001/2002 (2).

Pig husbandry is an integral component of rural economy in Nepal, which is also linked with socio- cultural activities of rural farmers especially in Tharu ethnic group. Pigs population in Nepal is 935,067 with the distribution 0.22 per household (6). The population of pig and duck in 24 JE endemic districts is 358,850 and 331,646 respectively. Abortion has been reported as one of the most common problem in the pigs. The total numbers of 286 outbreaks of abortion were reported from 2000 to 2004 in Nepal (6). Similarly 43 outbreaks of pig abortion were reported in 2005 (Personal communication with Veterinary Epidemiology Center). In 2001, 45,213 pigs were vaccinated with live attenuated JE virus vaccine in 22 districts of Nepal (7). Limited epidemiological studies have been carried out on JE in animal in the country. Central Veterinary Laboratory (CVL) has been launching JE investigation program since 2003 to study the sero status of JE virus in susceptible animal, which could speculate epidemiological information for the strategic control of JE in the country. This study was technically and financially supported by Australian Animal Health Laboratory (AAHL) and Crawford Training Fund, Australia.

Methodology

A total 633 serum samples were collected randomly from non vaccinated pigs, ducks and horses covering 19 districts during September 2003 to August 2005. Number of samples collected from pigs, horse and ducks were 534, 25 and 74 respectively (Table 3). These samples were stored at –20°C at CVL until use. Out of 19 selected districts, 16 were JE endemic and 3 non-endemic area (Kathmandu, Lalitpur and Bhaktapur). Out of 633 samples, 440 samples were inactivated at 56°C for 30 minutes and serum vials were disinfected by wiping with citric acid (0.01 M) and were sent to AAHL. The remaining (193) samples were tested at CVL. Competitive Enzyme-Linked Immunosorbent Assay Test (8) was used for antibody detection at both laboratories. In brief, 96 polyvinyl well plates were coated with JE antigen (AAHL). The test sera were added at 1 :10 dilution and JE-989 monoclonal antibody (Trop Bio, USA) was added to serum in ELISA plate.

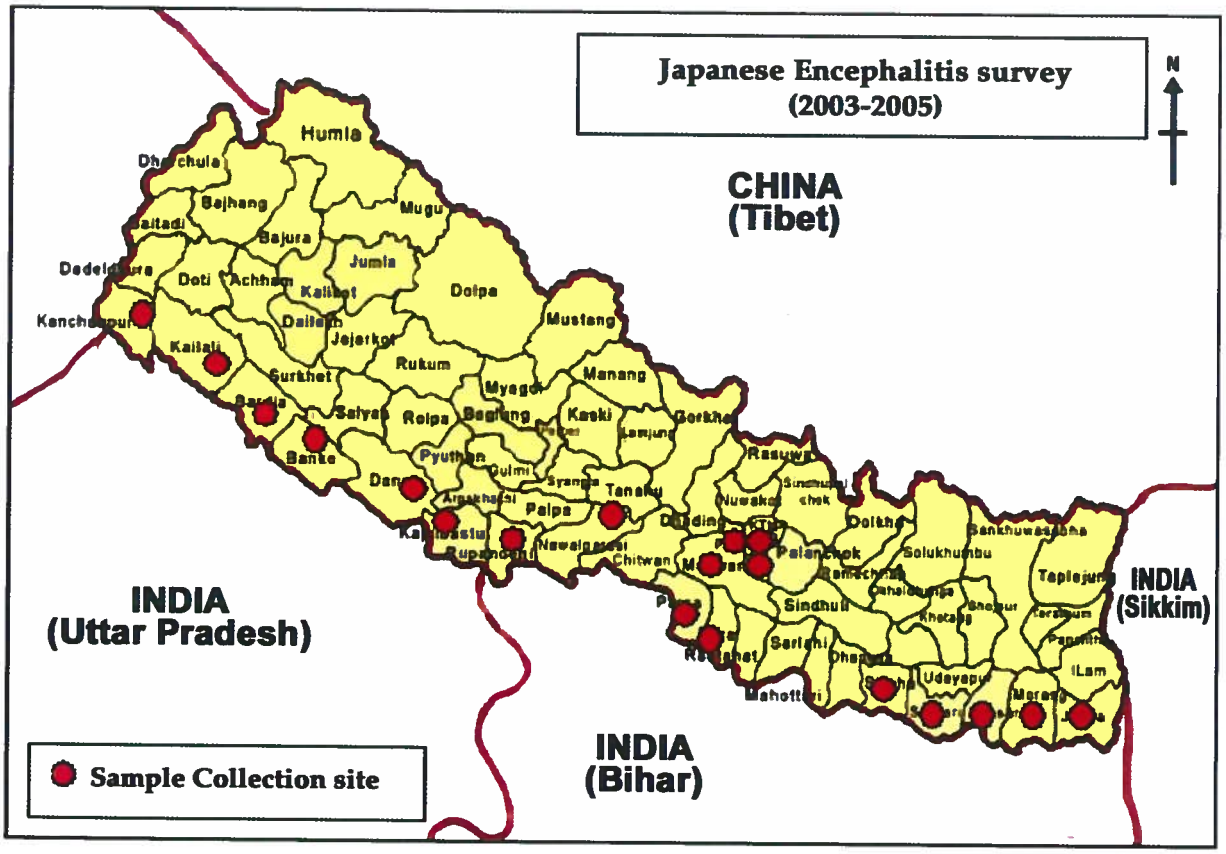
Jackson goat anti-mouse horseradish peroxidase was used as conjugate. Tetramethyl-benzidine (Sigma) was used as substrate. Sulphuric acid (1M) was added to stop the reaction. Optical densities were recorded using microplate photometer at 450 nm wavelength. Resultant optical densities were converted to percentage inhibition relative to a normal pig serum control. The sera resulting more than 40% inhibitions were considered as positive where as less than 40% were considered as negative.

Result

The results of C-ELISA in 19 districts of 5 region of Nepal are presented in Table 1. Out of 633 tested sera, 53.31% were found positive for JE antibodies (IgG and IgM) whereas the rest were negative. Sero- prevalence of JE in pigs, horses and ducks was determined 57.62%, 68.62% and 21.62% respectively (Table 2). Geographical distribution of JE in Far West, Mid West, Western, Central and Eastern regions was 80.26%, 37.38%, 35.41%, 56.72% and 59.56% respectively.

Discussion

In this study, uniform number of samples could not be collected from different geographical location. Meanwhile collected samples were not statistically significant. However this study confirmed presence of JE virus in pigs, horses and ducks in Nepal. In previous study, 36.3 % of pigs and 20% of ducks were positive for JE when 91 pigs' sera and 40 ducks' sera collected from 11 endemic districts of the country were tested (9).



In this study, the highest percentage of sero-positive cases in pigs was observed in Far Western Region of Nepal, which is directly co-related with the incidence of JE in human being (2). Highest number of cases and deaths was recorded 434 and 64 in Kailali in 2005 (Personal communication with Epidemiology and Disease Control Division). According to the Health Authority of Nepal, the incidence of JE in human being was higher in Dang, Kailali, Banke, Bardiya, Rupandehi, Kanchanpur, Kapilbastu, Morang and Sunsari in comparison to the other endemic districts. Sero-prevalence of JE in pigs was found 100% in Kanchanpur, Dang and Kapilbastu. However numbers of samples tested in this study do not represent statistically the total population of pigs in these districts. The sero-prevalence of JE was found 91.42 % in pigs in Lalitpur, which is a non-endemic district. However the incidence of JE in human being in Lalitpur is very low as compared to other districts which may be due to the well-organized pig farming and good hygienic status of people. Meanwhile pig farms are located far from human inhabitation in urban areas. In contrast, the people living in the rural area of highly endemic districts live very close to their pig farms due to the socio-economical factors. JE is principally considered as a disease of rural areas where the vector mosquito lives and grows in a close association with pigs. In pigs, clinical symptoms are not a feature of JE but abortion, stillbirth, abnormal fetus and infertility are closely associated with JE infection (10). In a survey conducted by CVL in 2004, abortion was

recorded 40% and still birth was found 30% in a pig farm located in Lalitpur district. All 17 samples collected from this farm were JE positive. High percentage of positive result in Lalitpur district may be associated with JE infection in pigs because other clinical symptoms rather than abortion were not observed in affected pigs. Only two sera collected from Parsa district were negative.

In this study, limited pig's serum samples were collected and tested only from 18 districts therefore the result of this study may not reflect the whole situation of JE in the country. However, the results of this study indicate that the prevalence of JE virus infection in pigs in 15 endemic districts and 2 non-endemic districts (Kathmandu and Lalitpur) has been technically justified. The sero- prevalence of JE in Banke district was comparatively low (29.89%) which does not directly correlate with the incidence of JE in human in Banke district.

Horses are being used as important means of human transportation mostly in Southern district of Nepal. Only 25 horse sera were collected from 4 endemic districts out of which 41.93% were found positive. In horse, clinically JE has not been reported in Nepal to date. Confirmatory laboratory diagnosis of horse suspected for encephalitis is needed to confirm JE infection. Although, horses do not play any role for the transmission of JE to human, the sero-prevalence of JE in these animals was studied first time in Nepal.

In ducks, 74 sera were collected from seven JE endemic districts of which 21.62% were found positive. Surprisingly all duck samples collected from Banke, Parsa and Rupandehi were found negative. It was reported that 20% of duck sera were positive (9). Although the number of collected sera from ducks were very less in comparison to the duck population in endemic districts, this result is a bit similar to previous finding (9).

Recommendation

Further sero-epidemiological study of JE is needed in depth by collecting statistically significant number of samples representing the population of pigs and ducks in endemic as well as non-endemic districts. Diagnostic facility for JE virus isolation should be introduced in Nepal for confirmatory diagnosis of *Favivirus*. Production of JE vaccine in the country and mass vaccination of people and pigs living in endemic districts would be the best JE disease control strategy in Nepal. Collaborative researches with international organization are highly recommended.

Acknowledgement

Author would like to acknowledge AAHL and Crawford fund of Australia for providing technical and financial support to perform this study in Nepal. My sincere thank goes to Dr. Peter Daniels and Mr. Ross Lunt of AAHL for their technical guidance and moral support during this study. I would like to give my gratitude to Dr. Rebati Man Shrestha, Chief Veterinary Officer of CVL and my colleague who were involved in JE investigation program. I highly appreciate the help provided by Dr. M.B. Bista, Director of Epidemiological Division. Finally I highly acknowledge World Organization for Animal Health as well as Center for Disease Control and Prevention for their support.

References

Khatri, I.B., Joshi D.D. and Pradhan, T.M.S. (1981). Epidemiological Study of Viral Encephalitis in Nepal. *J. Inst. Med.*, 14 : 133-144.

Epidemiology and Disease Control Division (2005). Annual Report 2002 and 2003 pp 22-31. Epidemiology and Disease Control Division, Kathamandu, Nepal.

Seghal, P.N. (1989). Control of Epidemics like Japanese Encephalitis and Meningococcal Meningitis Development of Epidemiological Surveillance for Prevention and Control of Epidemics in Nepal. Assignment WHO Report 17, january-27 February 1989.

Bista, M.B., Banerjee M.K., Singh, S.H., Tandan, J.B., Kim, M.H., Sohn, Y.M., Ohrr, H.C., Tang, J.L. and Halstead S.B (2001). Efficacy of single dose SA 14-14-14-2 vaccine against Japanese encephalitis : a case control study. *Lancet* 358 : 791-95.

Agribusiness Promotion and Statistical Division (2003). Statistical Information on Nepalese Agriculture pp 5-7. Agribusiness Promotion and Statistical Division Ministry of Agriculture and Co-operatives, Singh Darbar Kathmandu, Nepal.

Veterinary Epidemiology Center. (2004). Annual Epidemiological Bulletin January-December 2004, pp-128, Veterinary Epidemiology Center, Directorate of Animal Health, Kathmandu, Nepal.

Pant, G.R. (2004). Japanese Encephalitis Program at Central Veterinary Laboratory in Nepal.Annual Technical Report (2003-2004), pp 97-106 Central Veterinary Laboratory, Kathmandu.

Williams, D.T., Daniels, P.W., Lunt, R. A., Wang, L. F., Newberry, K.M., Mackenzie, J.S. (2001). Experimental infection of pigs with Japanese encephalitis virus and closely related Australian falvivirus. *Am. J.Trop.Med.Hyg.*65 : 379-87.

Joshi, D.D. and Gaidamovich S. (1981-1982). Serological surveillance of virus encephalitis in Nepal. *Bulletin of Veterinary Science and Animal Husbandry Nepal*, 10&11 : 8-12.

Joo, H.S. and Chu, R.M. (1999). Japanese B Encephalitis, Disease of Swine 8th Edition (Edited by Barbara E. Straw, Sylvie D'Allaire, William L Mengeling and David J. Tailor) pp 173-178 , Black Well Science Limited, London.

ANNEX

Table 1 : Summary of JE, C-ELISA result according different regions (2003 - 2005)

Region	District	Tested samples	Positive samples	Positive %	Mean %
Far-Western					
	Kailali	70	55	73.03	
	Kanchanpur	06	06	100.0	
	Sub-total	76	61		80.26
Mid -Western					
	Banke	93	28	29.89	
	Bardiya	09	07	64.28	
	Dang	05	05	62.50	
	Sub-total	107	40		37.38
Western					
	Kapilbastu	15	04	26.66	
	Nabalparashi	44	22	50.00	
	Rupandehi	37	08	21.62	
	Sub-total	96	34		35.41
Central					
	Makwanpur	75	42	56.00	
	Bara	16	05	31.25	
	Parsa	10	02	20.00	
	Kathmandu	31	13	41.93	
	Bhaktapur	04	03	75.00	
	Lalitpur	35	32	91.42	
	Sub-total	171	97		56.72
Eastern					
	Sunsari	78	42	53.84	
	Morang	44	32	72.72	
	Jhapa	21	09	20.45	
	Saptari	24	14	58.33	
	Shiraha	16	12	75.00	
	Sub-total	183	109		59.56
Grand total		**633**	**341**		**53.87**

Table 2 : Percentage of positive cases according to the species of host (2003-2005)

Species of host	Tested samples	Sample positive	Positive %
Pig	534	308	57.67
Horse	25	17	68.00
Duck	74	16	21.62
Total	**633**	**341**	**53.87**

Table 3 : Sero-prevalence of JE in pigs, horse and duck in different regions (2003 - 2005)

District	Pigs samples			Horses samples			Ducks samples		
	TT	TP	PP	TT	TP	PP	TT	TP	PP
Kailali	50	46	92	12	6	50	8	3	37.5
Kanchanpur	6	6	100						
Banke	69	19	27.53	9	9	100	15	0	0
Bardiya	9	7	77.77						
Dang	5	5	100						
Kapilbastu	1	1	100	14	3	21.42			
Nabalparasi	44	22	50						
Rupendehi	23	8	34.78				14	0	0
Makanwanpur	75	42	56						
Bara	12	4	33.33				4	1	25
Parsa	2	0	0	4	2	50	4	0	0
Kathmandu	31	13	41.93						
Bhaktapur	NC	NC					4	3	75
Lalitpur	35	32	91.42						
Sunsari	78	42	53.84						
Morang	33	26	78.78				11	6	54.54
Jhapa	21	9	42.85						
Saptari	24	14	58.33						
Siraha	16	12	75						
Total	**534**	**308**	**57.67**	**25**	**17**	**68.00**	**74**	**16**	**21.62**

Note : NC= Samples not collected or available
TT= Total tested
TP= Total positive
PP= Positive percentage

* This paper was presented in International Symposium on Emerging Zoonoses held from 22-24 March, 2006 Atlanta, Georgia, USA organized by OIE and CDC.

Khari Disease Investigation : A Report

Dr. Rebati Man Shrestha
Chief Veterinary Officer
Central Veterinary Laboratory
Dr. Raju Gautam
Veterinary Officer
Regional Veterinary Laboratory, Dhangadhi

1. Introduction

Khari is a chronic debilitating disease. It principally affects the buffaloes and it has been prevailing in the two districts of the far western region; Baitadi and Darchula. However, the disease has also been observed in cattle. It has caused serious financial loss to the farmers of these districts.

2. Objectives

2.1 Short term objectives

- To carryout epidemiological study of the disease.
- To conduct drug trials.
- To strengthen the diagnostic capability of the RVL, Dhangadhi.

2.2 Long term objectives

- To find the etiology of the disease.
- To develop effective and reliable therapeutic regime and suggest possible control measures.

3. Materials and methods

3.1 Materials

Over hundred samples of different types were collected from among the khari disease affected animals of Darchula and Baitadi. The samples included EDTA Blood, Blood smear, Serum, Hoof dust, Skin scrapings, hay, grass, soil etc. Many of the samples were tested at the RVL and those tests which could not be performed at the RVL were sent to the CVL and other reference laboratories of the country. Details about different samples are presented in table 1.

Table : Type and the number of samples collected

S. N.	Type of Sample	Number
1	EDTA Blood	32
2	Blood smear	62
3	Serum	119
4	Skin Scraping	20
5	Hoof dust	14
6	Soil	3
7	Hay	2
8	Fodder	1
9	Grass	4

3.2 Methods

3.2.1 Epidemiological survey

Preliminary survey of the disease situation was conducted using a set of questionnaire. Epidemiological information was also collected during field visit for sample. It was found that the adult animals were most commonly affected i.e. animals of the age 8.7 years and above were more frequently affected. Females were found to be affected more than the male individuals. However, occurrence of the disease in younger group of animals or male was not uncommon. The disease was recorded in a male buffalo calf aged one year and a three year old male calf. The incidence of the disease in buffalo population was found to be 80.35 % and in cattle were 19.64% of the total animals under investigation. It has also been found to occur in dry animals, the survey result of which is presented in table 1.

Table 1 : Epidemiological status of the Khari disease in Bovine Population

S. N.	Species	Sex		Total	Average age
		Male	F		
1	Buffalo	1	44	45	8.7 year
2	Cattle	1	10	11	

The survey on feeds and feeding reveals the widespread use of hay made from locally available grasses. Hay (grass straw), which is locally known as Gajio is the principal source of food to the animals of these two districts during the lean period (October – May). Various types of grasses used in preparation of hay are cut and dried during September - October and stored by staking in the form of a tower. The inclusion rate of different grasses used in making hay is presented in table 2.

Table 2 : Inclusion of local grasses used in making hay

S. N.	Local Name	Scientific Name	Percentage composition in hay
1	Atharne	Heteropogon contortus	60%
2	Ghorade	Chrysopogon	30%
3	Babyo and others	Eulabopsis and others	10%

3.2.2 Laboratory examinations

3.2.2.1 Mycological examinations

The hoof scrappings were collected from the buffaloes showing symptoms and signs of khari disease. These samples were kept in sterile Petri dish maintained inn cool pack and brought to the laboratory for the purpose of fungal culture. The different culture media used in the study were Sabourad's dextrose agar and other selective media

3.2.2.2 Haematological examination

The specimen of blood from the ailing animals were collected in ethylene diamine tetra acetic acid and maintained in cold and subjected to study the various parameters; Hb content, PCV, ESR and differential leukocyte count. The Hb content was estimated by Sahli's method, PCV with microhaematocrit method, DLC with Neubaeur's countring chamber and ESR with Wintrobe's method. The biochemical assay of sera was tried at RVL with the use of commercial kits.

3.2.2.3 Parasitological examination

The parasitological study was conducted to explore the involvement of ectoparasites in of development of the disease. The skin scrapings were examined with the use of KOH method.

3.2.2.5 Examination of hay and soil

The samples of the locally available grass used in making hay and the samples of soil were collected from the study area and sent to various laboratories to study the proximate principles as well as physical property and the mineral content respectively. Samples of a few grasses occasionally used in making hay were also collected and sent to the Natural Products Research Laboratory for identification. Hay sample was also forwarded to the Animal Nutrition Division of the Nepal Agriculture Research Council (NARC) for proximate analysis. Similarly, soil samples were also collected and sent to soil laboratory for testing of various parameters. Also the soil test record of the Darchula district was obtained from the Regional Soil Laboratory, Dhangadhi. Theywere collected from cultivable land as well as the grazing land or the land from where the grass is obtained for making hay.

3.2.2.6 Drug trial

A study pertaining to drug trial was conducted to provide a line of treatment to the farmers. The proposed line of treatment was formulated jointly by RVL, Dhangadhi and CVL, Tripureshwor. It was studied by dividing the ailing animals in six different groups based on the type of treatment regimen as follows.

Group 1 : Ivermectin @ 1ml/50 kg. body weight, two injections at monthly interval.

Group 2 : Minamil at the rate of 50 gm. per day for 15 days per month to be used for two months.

Group 3 : Ivermectin @ 1ml/50 kg. body weight two injections at monthly interval Plus Minamil at the rate of 50gm per day for 15 days in a month for two months period.

Group 4 : Minamil @ 50 gm per day for 15 days per month for two months and Tonophosphan 10 ml per animal, 2 injections at 15 days interval.

Group 5 : Ivermectin as described for G1 and Tonophosphan as for G4

Group 6 : Ivermectin, Tonophosphan and Minamil at the dose rate described above.

4. Result and Discussion

4.1 Result

The mycological study of hoof scrapings revealed the presence of Blastomyces spp, Candida spp and Absidia spp of molds. The haematological test results of various parameters of blood samples are presented in table 3. However, the biochemical estimation of serum calcium level done at RVL, Dhangadhi was supposed to be erroneous giving the results ranging from 4.5 - 22 mg/dl.

The ectoparasitic examination of 20 skin scrapings comprising 18 samples from buffaloes and two samples from cattle revealed altogether 16 positive samples; 15 samples from buffalo and one sample from cattle for the presence of Sarcoptes spp of mites and four samples were found negative. The results of proximation analysis of hay are presented in table 4 and the chemical compositions of the soil samples are presented in table 5 and 6.

Table 3 : Result of various haematological parameters

S. N.	Haematological parameters	Total samples	Average value	Range
1	Hb	25	6.56 g/dl	5.2-8 gm/dl
2	PCV	14	35%	
3	ESR	5	43.6 mm/hr.	28-65 mm/hr.
4	Lymphocyte	36	44%	35%-67%
5	Neutrophil	36	50%	26%-58%
6	Monocyte	36	4%	2%-8%
7	Eosinophil	36	2%	

Table 4 : Result of proximate Analysis of Hay and Grass samples

S. N.	Sample type	DM %	CP %	NDF %	ADF%	Lignin
1	Hay	88.63	6.14	82.69	67.13	9.17
2	Hay	86.15	11.05	56.29	55.19	29.31
3	Grass	65.87	4.06	62.68	55.15	9.76

Table 5 : Result of the Phosphorus test of Soil, collected from the Khari affected area of Darchula, (Tested by Soil Lab, Dhangadhi)

S.N.	Name of the farmers	Address	Place of the soil collection	Phosphorus contents		Remarks
				ppm	Kg/h	
1.	Man singh Mahar	Darchula, Banjh	Field	0.1120	51.29	Medium
2.	Man singh Mahar	" "	Garden	0.117	54.04	Medium
3.	Man singh Mahat	" "	Fodder soil (upland)	0.049	22.9	Low
4.	Man Singh Mahat	" "	Animal Shed	0.123	56.33	High
5.	Lokmani Thaguna	" "	Garden	0.060	27.48	Low

Table 6 : Results of the Soil Examination at the Regional Soil Lab., Dhangadhi

Name of the farmer	Address	pH	O.M (%)	N_2 (%)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (ppm)	Fe (ppm)	Mg (ppm)
Soil from grazing land	Rodidewal, Baitadi	8.8 (Salty)	3.71 (Medium)	0.185 (Medium)	43.3 (Medium)	77 (low)	10 (optimum)	1	-
Birendra Singh	Rodidewal-8, Baitadi	8.8 (Salty)	2.77 (Medium)	0.138 (Medium)	41.9 (Medium)	85 (Low)	25 (optimum)	1	-
Harka Singh Mahara	Khalanga-9, Darchula	7..28	2.45 (Medium)	0.122 (Medium)	50.01 (Medium)	365 (High)	25 (optimum)	-	2.5 (very low)
Lal Singh Karki	Shankarpur-9, Dharchula	5.96 (acidic)	2.69 (Medium)	0.134 (Medium)	46.02 (Medium)	270 (Medium)		-	10 (very low)

The result of aforementioned treatment regimen suggested only a slight improvement in those animals under Minamil treatment i.e they were able to move around with ease than before, however the skin lesion remained unchanged. Similarly the animals which were treated with Ivermectin showed disappearance of skin lesions, and encouraging regrowth of hair in the affected region but without the improvement of the emaciated body condition.

Much better results were obtained when Ivermectin and Minamil were used in combination. This treatment regime supplemented with a few quantity of concentrate feed to the animal gave encouraging result with not only the improvement of the skin lesions but also the body condition and slight improvement in the hoof furrow. Very encouraging result was recorded in animals which were given all of the aforementioned drug regimen revealing recovery of the animals from lameness, improved skin condition and body condition, and appetite.

5. Discussion

The epidemiological finding in the present study is greatly different from earlier observations and findings, which reported the disease condition to occur only among adult milking buffalo population. In contrary to the finding of Singh et.al (1996-97) who reported the incidence of the disease only in milking adult buffaloes, considerable number of dry buffaloes including male and cattle were also found to be affected by the disease as is evident from table 1.

The presence of Blastomyces spp, Candida spp and Absidia spp of molds in hoof scrapings suggests the involvement of fungus as one of the causative agents of khari disease. In general, the animal sheds were found to be ill-ventillated, damp and with the least access of sun light, an attribute suggesting the congenial environment for mold growth. Similarly, chronic course of the disease, debilitating condition of the animals suggest for the fungal infestation. Therefore, the clinical examination of ailing animals and examination of their environments suggested for systemic fungal infestation.

Similarly, the haematological parameters revealed no significant changes except for the Hb content and ESR value. Hb content was found to be subnormal and the ESR was recorded to be abnormally high. The high ESR value may represent the chronic nature of the disease. However, the values of ESR have not been studied and compared in the light of urinary problems, or other neoplastic changes in the present study. Biochemical estimation of serum for calcium level

attempted at RVL, Dhangadhi suggested the values to range between 4.5 - 22 mg/dl. However, the estimation method seemed to be erronius and therefore, referred to other laboratory for verification of the present finding.

The extoparasite examination of skin lesions is consistent with the finding of Ratala and Singh (1993-94) and Singh et.al (1995-96) in so far as infestation of diseased animals with mange mites. However, it is contrary to our finding of *Sarcoptes* spp of mange as compared to their finding of *Psoroptes* spp mange in their reports. Moreover, it can be inferred that the skin lesions present in khari affected animals may also be attributed to the infestation of mange mites due to either species fo the mange. Also, the presences of mites may be responsible for, to some extent, anaemia in the ailing animals. However, it may not be the soul cause of the debilitated bodily condition or the cause of the foot lesions.

The availability of crude protein in the straw used for feeding animals is very low as is evident from the result of proximate analysis given in the table above and the finding is consistent with the reports Dabadghao & Shankarnarayan (1970) in India for Heteropogon contortus.

Heteropogon contortus is identified as spear grass with stem 30-150 cm long. The root has a diuretic property and the awns injure the mouth and skin. Awn oil is given in asthma. The plant also contains Myo-inositol, galactinol and raffinose. It can be a good hay if cut before flowering containing DM- 93%, CP- 6%, and CF- 37%. When fresh the grass recorded to contain CP- 9% and CF- 30%. It can however, be noted that Dabadghao & Shankarnarayan (1970) in India found the crude protein content of a Heteropogon community was 5 percent untreated and 5.8 percent when treated with nitrogen. The digestibility of the protein is however, low. The grass is reported to be palatable in the early vegetative stage, but unattractive as it matures (L.Beauv. ex Roem. and Schult). The phosphorus figures as percentage of dry matter is reported to range from 0.09-0.15 which is far below the requirement of the dairy cattle, and the calcium content is barely sufficient, ranging from 0.23 to 0.30 as percentage of the dry matter.

It has been said to cause Birdsville disease with symptoms of high stepping with forelegs and holding the head high. The hind limbs swayed and dragged the toes of their hind feet. The most severely affected keep their hind legs abducted and if exercised, staggered backwards and fell over. (Aust. Vet-J. 1983, 60 :316-7- cited by K.Narayana et *al* 2003 in Poisonous and Medicinal Plants).

The Natural Products Research Laboratory has also given the test of toxicity of the hay. It has reported that the alcohol extract of the hay used for feeding the livestock subjected to acute toxicity test on mice caused death of 25% of the mice at the rate of 500mg/kg body weight, whereas, no mortality was observed when administered at 300mg/kg body weight.

The examination of soil samples revealed that the phosphorus content of the soil is comparatively low, which co-relates with the phosphorus content of the grass available in the aforementioned districts. Similarly, calcium content of the soil is optimum in all the soil samples examined. This also explains the lower content of phosphorus. The amount of magnesium and potassium is also comparatively low. The organic matter of the soil along with other important micronutrients like nitrogen content is also insufficient.

References

Bhatt K.L., An Experience with Khari Disease in Baitadi District, Quarterly Epidemiological Bulletin, April 2004-June 2004, Vol.11, Number 2, PP 57-62.

Gongol G.N., The Epidemiology of Khari Disease in Nepal during the period of 1998-2003, Quarterly Epidemiological Bulletin, April 2004-June 2004, Vol.11, Number 2, PP 51-56.

Narayana K., Pradeep S. Kundur., Usha N., Sridhar N.B., Poisonous & Medicinal Plants, first edi. 2003, Jayashri Publications 161, 4th Cross, Lower Palace Orchad, Bangalore 560003. India.

Ratala D.R., Manandhar P., Pant G.R., Preliminary study of Khari Disease of Buffalo in Baitadi District. Bull. Vet. Sc.& A.H, Nepal Vol 19-20, 1990-1992.

Ratala D.R., Singh U.M., Study of Evaluation of Ivermectin Injection Against Khari Disease, Proceeding of the Fifth National Veterinary conference of NVA.

Singh U.M., Shrestha S.P. (1996) Study of Khari Disease in Buffaloes of Baitadi and Darchula District of Nepal, Proceedings of Ist Nat. Liv./Fish Rs. Workshop 1996, PP 179-184.

Investigation of kid mortality in goats of the far western region

Dr. Raju Gautam
Veterinary Officer
Regional Veterinary Laboratory, Dhangadhi

1. Background

Goat rearing is an intregal component of subsistance agriculture system in Nepal. In recent years, goat rearing is gaining popularity in various districts of far western region of the country. The present goat population of the region is projected as 616,115, which constitute 9% of the total goat population of the country. Goat is the second largest source of meat in our country rating next only to buffalo and contributes about 19.47% of the total meat production in the country (MOAC, 2002/2003). At the present growth rate of goat population is 7% per annum with the addition of around 40000 goats every year. However, together with its fast population growth, goat rearing is facing new challenges in management, heath and disease control.

Among the various constraints responsible for the diminished productivity in livestock sector, disease is generally agreed as one of the most important factors. With the progress in goat farming in the region and steady shift in farming system from traditional subsistence goat farming practices toward commercialization, many problems are arising. One of the problems that have emerged as relatively important among others is the increased mortality of kids below six months of age. Also in the past, the problem of kid mortality had been reported from various farms of different districts, and also from the goat development farm of Budhitola. In order to scientifically address this problem, an investigation on kid mortality was initiated in the two far western districts of the region namely Kailali and Kanchanpur.

2. Objectives

2.1 Long Term objective

To identify the major causes of kid mortality and disseminate the information generated to the field veterinarians, technicians and needy farmers.

2.2 Short Term objective

- To assess the prevalence of infectious and non infectious causes of kid mortality.
- To study the epidemiological pattern of kid mortality.
- To strengthen the diagnostic capability of RVL, Dhangadhi.

3. Materials and Methodology

3.1 Materials

Blood samples in ethylene diamine tetra acetic acid, nasal swabs and faecal samples.

3.2 Methodology

3.2.1 Site selection

Goat rearing farmers for the investigation program from Kailali and Kanchanpur were selected by an interaction program organised separately for the two districts. A total of 10 farmers Six from Kailali and four from Kanchanpur were selected and were distributed with the questionnaire format developed by the RVL, Dhangadhi to be filled on monthly basis. Timely visit by the RVL staff was made to monitor the programme and collect desired samples from the dead kidswhich were initially sick for laboratory investigation.

3.2.2 Epidemiological investigation

The information obtained through the questionnaire over two years of the investigation period with the selected goat raising farmers revealed that on an average 34.8 kids were born per farm in a year. Mortality of female kids during pre-weaning period was found greater than the male kids.

Table 1 : Pattern of Kid Mortality

S. N.	Pattern	Number
1	Number of kids born	622
2	Number of Male kids born	328
3	Number of female kids born	294
4	No. of male kids death	46
5	No. of female kids death	58

The pre-weaning death of kids was found to be related with the type of birth. Highest percent of death was recorded in the kids who were born as triplets followed by twins. Least number of mortality was recorded with kids born as single (table 2).

Table 2 : Kid Mortality in Association with the Type of birth

S. N.	Type of birth	Number of kids	Number of death	Percent
1	Single	276	11	3.98
2	Twins	310	37	11.93
3	Triplet	36	12	33.33
Total		**622**	**60**	**9.64**

Rate of kid mortality also appeared to differ with the season, with highest mortality recorded during the summer/rainy season, followed by winter compared to lower mortality observed during the spring and the autumn seasons. This finding is in agreement with the report of Aryal *et al* (2001). The reason for higher mortality during rainy and winter season could be attributed to the adverse weather condition imposing stress upon the kids rather than the occurrence of any specific disease condition. It is also likely that greater numbers of kids are born during these two seasons compared to other two seasons which witness lower birth rate.

Table 3 : Kid Mortality in relation to season

S. N.	Season	Number of death	Percent
1	Winter (Marg-Magh)	17	28.3
2	Spring (Falgun-Baisakh)	8	13.33
3	Summer/rainy (Jestha- Shrawan)	29	48.33
4	Autumn (Bhadra–Kartik)	6	10

Mortality was observed highest during the period of 15 days to 1 month of age in the kids. Next was the period between 1 to 3 months of age (table 4). This observation is contrary to the findings of Aryal et al (2001) that reported highest mortality in kids less than 15 days of age revealing a decreasing trend in the mortality with the increasing age of kids.

Table 4 : Mortality associated with the age of kids

S. N.	Age of Kid	Number dead
1	Less than 15 days	11
2	16 to 30 days	24
3	1 to 3 months	16
4	Above 3 months	9

3.2.3 Laboratory investigation

Various types of samples were collected during field visit as per the clinical finding and they were examined applying parasitological, haematological and microbiological techniques.

4. Result and Discussion

4.1 Results

4.1.1 Parasitological examination

Most of the faecal samples collected from the kids under investigation were found infested with one or the other kind of nematodes. Out of 26 faecal samples examined so far 21 were positive for different nematodes. However, fluke infestation was not recorded in any of those samples. Major round worms identified belonged to Strongylus, Strongyloids and Trichuris.

4.1.2 Haematological Examination

Haematological examination of the clinically ill kids was carried out to assess change in major blood parameters such as PCV, Haemoglobin, and blood protozoan parasites. Findings are presented in the table below :

Table 5 : Result of Haematological Examination

S. N.	Test	Average value
1	Haemoglobin	9.1g/dl
2	PCV	20.4%

Microbiological Examination

Nasal swabs were collected from clinically sick kids showing signs of pneumonia for microbiological examination. Bacterial identification was done on the basis of cultural characteristics of the colony, and gram's staining. Major bacteria identified were *Pasturella* spp and other Gram negative rods and *Staphylococcus* spp, *Streptococcus* spp and other Gram +ve cocci.

4.2 Discussion

The present finding about pattern of kid mortality is contrary to the observation made by Neopane (1996), Singh et *al* (1990), Gebrelul et *al* (1994) and Aryal et *al* (2001) that reported higher survival rate of female kids. However, Mittal (1976), Majumdar et al (1980) and Sharma et al (1981) reported the non significant effect of sex on survival rate of kids during pre-weaning period.

It is observed that kid mortality is positively related with the type of birth, evidencing that those born as triplets or twins had lower rate of survival compared to kids born single. Higher mortality was observed during the rainy and winter season and the common diseases identified to cause death were pneumonia, diarrhea, Anuria, Paralysis etc. Since, highest mortality appear to be caused due to pneumonia of infectious nature i.e. either parasitic or bacterial; there is ample room for decreasing the rate of kid mortality by better management and adoption of proper health management and parasitic control program

The finding for type of kid mortality is in agreement with the report of Aryal et al (2001), Sharma et al (1981), Prasad (1983) and Singh et al (1990) who reported that singles had higher survival rate than the twins or the triplets. Mortality rate of 33.33% presented here, which appears to be exaggeration of the finding may be due to small size of the triplets observed, nevertheless justifies the increased mortality in triplets compared to twins and singles due to limited nursing opportunity with the increased litter size. Still it requires further investigation to reflect the exact or the reliable percentage of the deaths occurring in the kids born as triplets. However, the overall percentage of kid mortality was observed to be well below 10 percent which is as the expected value in well managed farms.

Conclusion

Table 6 reveals the various causes of kid mortality among which the most common cause of death of a kid was identified as pneumonia, followed by diarrohoea, paralysis and anuria. Pneumonic conditions in the kid were identified as infectious and parasitic depending on the clinical symptoms and presence or absence of fever.

Table 6 : Diseases/conditions responsible for kid mortality

S. N.	Type of disease/condition	No. of death
1	Infectious Pneumonia	18
2	Vermiceous pneumonia	13
3	Paralysis	7
4	Anuria	6
5	Diarrhoea	12
6	Others	4

References

Statistical information on Nepalese Agriculture, 2002/2003, MOAC.

Aryal et al, NARC, 2000. A study on goat and sheep health with particular reference to kid mortality.

Study on Sukharia disease of cattle in Saptari district of Nepal

Dr. Sada Nand Deo
Senior Veterinary Officer
Dr. Keshab Prasad Sah
Veterinary Officer
Regional Veterinary Laboratory, Biratnagar

Abstract

Lantana poisoning has been taking a heavy toll of cattle year after year. A study was carried out in different villages of Saptari district in the fiscal year 2062/063 to find out etiological epidemiology of Sukharia disease in cattle. Altogether 35 cattle were selected on the basis of history and clinical findings. Different types of samples; blood, serum, urine, skin scraping, samples of local fodder and necropsy samples were collected and accessed for various tests. Hamatological study revealed normal blood parameters except for low haemoglobin contentt and neutrophillia. Most of the urine samples were positive for protein. Biochemical estimation revealed the serum level of total protein, total bilirubin, and SGOT above the normal range. Necropsy examination showed enlarged liver and gall bladder. The suspected Lantana camara plant examined at Natural Products Research Laboratory, Department of Plant Resources, Thapathali, Kathmandu was positive for the toxin triterpene acid. The history, clinical findings, and laboratory results as well as toxic principle present in Lantana camara plant provides strong base to confirm the plant as the cause of Sukharia disease in cattle of the areas under study.

1. Introduction

Sukharia is a location specific disease of cattle characterized by dryness of skin, dry faeces in the form of pellets, general debility and death. The disease has been reported in cattle of Saptari district during the period of June-September for the last eight years. The cause of the disease remained uncertain despite of several studies. The disease is locally known as *Sukharia* due to the fact that the animals suffer from the dryness of the skin, constipation and dry pellet faeces. Because of the sloughing of the skin, this is also called *Wadarah* in local tounge. The disease has been reported in the north eastern part of Saptari district and the Village Development Committees (VDCs) affected are Ghoghanpur, Rupnagar, Bhardah, Baramajhia, Badgama, Bairba, Jagatapur, Mainakaderi, Lohajara, Madhawapur, Odraha/Kamalpur, Diman, Tirkaul, Phattepur, Portaha, Baluwa and Giothi according to the epidemiological data collected by District Livestock Services Office, Saptari. Cattle especially young are susceptible and show signs of depression and inappetence, constipation and dry faecal pallets, reddening and inflammation of skin, photosensitization of different parts of skin, dermatitis, necrosis, curling and sloughing of ear tip, extensive necrosis of skin even to loss of tail and hooves, yellowish discoloration of mucous membrane, reddish yellow colour urine In acute cases death occurs within two days whereas in

chronic cases death in 1-3 weeks primarily due to starvation, dehydration, and conccurrent infections.

2. Materials and methods

2.1 Clinical examination

The study was conducted in the fiscal year 2062/063 in different VDCs of Saptari district to investigate Sukharia disease in cattle. Formats were designed and history as well as primary records related to clinical findings were collected by direct interviewing with farmers and clinical examination of affected cattle adopting door to door survey system of selected sites. Altogether 35 horses were screened on the basis of history and clinical findings from areas having most of the cattle suffering from Sukharia disease (Table 1). Five control animals (apparently healthy cattle) were also selected.

Table 1 : Details of site and case selection

S. N.	VDCs	Total cases
1.	Bhardah	8
2.	Bairaba	6
3.	Jagatpur	6
4.	Badgama	10
5.	Kanchanpur	5
Total		35

2.2 Laboratory examination

- Collection of whole blood in EDTA for the estimation of Hb, PCV, TLC & TEC.
- Blood smears prepared simultaneously for blood parasite examination.
- Separation of serum for estimation of bilirubin, total protein and SGOT.
- Urine samples collection for examination of blood, ketone-bodies, pH, protein and glucose.
- Skin scrapings collection for isolation of fungus and examination of external parasite.
- Different varieties at different stages of *Lantana camara* plant collection for chemical analysis (to find out toxin triterpene acid).
- Samples of drinking water for estimation of arsenic content.
- P.M. examination to find out changes in normal organs.

Result and discussion

On the basis of history presented by farmers and clinical findings, cattle are the only species found to be affected by Sukharia disease. The affected cattle population includes mostly young with their age ranging from 6 months to 3 years with an average mortality of 8 percent which is in accordance with the statement of K. James who mentioned an outbreak of *Lantana camara* poisoning in cattle in which 10 out of 91 animals died.

In the present study as shown in Table 2, the average value of Hb, PCV and RBC count in Sukharia affected cattle was below normal value. However, percentage of neutrophils in the blood was above normal range. Blood parameters show anaemia and neutrophilia in affected

individuals. Anaemia may be due to chronic nature of the disease where as neutrophilia gives an indication of secondary bacterial infection through open skin wound.

The blood serum values of total protein and total (Table 3) were higher than the normal value which is suggestive of liver and kidney damage. Similarly the result of urine analysis (Table 4) showed proteinuria which is suggestive of Kidney damage. These findings justify the presence of hepatotoxin and nephrotoxin in the suspected local plant.

Table 2 : Blood examination

	Hb (gm %)	PCV (%)	Total count		Differential count			
			RBC million / cu.mm	WBC Thou. /cu. mm	N	E	L	M
Normal values	11.3	34	5.96	7.03	29	9	54	4
Control animals	9.2	27	5.02	7.06	32	8	56	4
Sukharia affected cattle	7.5	23	4.03	7.09	40	6	50	3

Table 3 : Blood serum examination

	T. Protein (gm / dl)	T. Bilirubin (mg / dl)	SGOT (μ / l)
Normal values	5.7-8.1	0-1.9	60-150 Avg - 105
Control animals	5.0	0.7	90
Sukharia affected cattle	5 cases- normal Others -↑	31 cases + ve (1.7 to 3.5)	120-220

Table 4 : Urine examination and analysis

	pH	Blood	Ketone bodies	Protein	Glucose
Normal cattle	7.9	-ve	- ve	- ve	- ve
Control animals	7.8	-ve	- ve	- ve	- ve
Sukharia affected cattle	7.6	2 cases +ve Other -ve	- ve	20 cases ++ve 9 cases +ve Others -ve	- ve

The examination of skin scraping, drinking water and blood smear revealed the absence of external parasite and fungus, arsenic content and blood parasite respectively. These are an indication of an absence of skin diseases caused by other factors and protozoan infestation.

Liver and kidney were the most affected organs found in post mortem examination of four cattle. The liver was enlarged, the gall bladder distended, and the carcass icterus. These are indication of *Lantana* plant poisoning.

The *Lantana camara* plant was sent to Department of Plant Resources, Thapathali, Kathmandu and on May 25, 2006, it confirmed the presence of toxin "triterpene acid" in *Lantana camara* plant.

organisms in CRD and other respiratory diseases. A study conducted by Dhakal 2002, on common poultry diseases suggests 18.20% prevalence of colibacillosis in the year 1999 and 10.99% prevalence in the year 2000. According to his finding, poor management, contaminated water and feed were the main sources of infection and heavy mortality seen in young chicks and growers.

2. Objectives

- To find out the prevalence of *Escherichia coli* in drinking water of poultry in western Chitwan.
- To find out the pH of water used in poultry industry.
- To find out the different types of drinking water sources used for poultry industry.
- To find out pathogenic effect of *E coli* isolated from water sample.

3. Materials and Methodology

A total number of one hundred and five water specimens from various sorces were collected in the present study. Those samples were taken in sterile syringe directly from water sources. All the samples were taken from western Chitwan region.

3.1 Site selection

For the purpose of present study, specimen of water was collected form its various sources from the western belt of Narayangadh municipality.

3.2 Sample Collection

Water samples were collected directly from the sources of water used for drinking poultry. All the possible aseptic condition was maintained and the collected samples were taken in the sterile syringe of 10 ml capacity.

Then the sample was taken to NAL Bharatpur for study. National Avian Laboratory Bharatpur was the main site of research in this study. However the works were performed in both NAL & VTH Rampur due to the limited source of lab facility and farm facility. The work in NAL was done in bacteriology unit.

3.3 Culture of water samples

All the collected samples were cultured in nutrient agar and MacConkey's agar. Positive growth with pink colony in Mac Conkey's agar media were suspected for *E coli* and were preserved in nutrient broth with 20% glycerin under refrigeration. Then the preserved sample (pink colony from MacConkey's) was further cultured in. EMB agar on which the organism produced characteristic green black colony with metallic sheen. Then it was further preserved for other biochemical tests.

3.4 Gram's staining

Smears of the culture were prepared on a clean grease free glass slide and they were fixed with flaming.The smears were stained with Gram;s staining technique as follows.

- Crystal violet for 60 seconds

- Washed under slow running tap water
- Gram's iodine for 60seconds.
- Washed under slow running tap water.
- Gram's decoloriser for 15 seconds.
- Washed under tap water
- Safranin solution for 60 seconds.
- Washed with tap water and allowed to dry and examined under microscope.

3.5 Biochemical tests

The Gram negative bacteria were subjected to catalase, oxidase and IMVP (Indole, Methyl red, Vogue Praskeur) test, motility test, citrate utilization test and Triple sugar iron (TSI) agar slant test.

3.5.1 Catalase test

For this test a drop of 3% H_2O_2 was taken in a slide and a loopful of bacterial colony was added over it. An effervescence of O_2 gas within few seconds indicated a positive reaction.

3.5.2 Oxidase test

For this test a piece of filter paper was taken and drop of oxidase reagent (1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride) was added to it, than test bacteria were streaked firmly over the filter paper with glass rod. A dark purple color along the streak line within 10 seconds was positive. But *E. coli* were oxidase negative so only negative sample were taken for further test.

3.5.3 Motility test

Motility test was performed by hanging drop method. For this test clear cover-slip was taken with a drop of distill water in the middle and a colony of bacteria to be tested was spread over that drop of water and observed under microscopic lens. Most of the *E. coli* was found as motilility positive.

3.5.4 Indole test

Materials required

- Peptone water
- Inoculating wire
- Kovac's reagent

Method

- A loopful of bacteria culture was taken with the help of sterilized inoculating wire loop and it was inoculated into a test tube containing 5 ml peptone water.
- It was incubated for 24 hours in 37^{0}C.
- 0.5ml of Kovac's reagent was added to the test tube next day.
- Indole positive sample gave red color whereas negative sample were colorless.

3.5.5 MR Test

Materials required

- MRVP media and methyl red, and incubator.

Method

- The sample to be tested was cultured in MRVP medium and incubated at 37^{0}C for 5 days. Two drops of methyl red reagent/ml broth was poured to the test tube. Appearance of red color was positive for MR test while appearance of yellow color was negative.

3.5.6 VP Test

Material required

Test tube, MRVP broth, Alpha- Naphthol, 40% KOH, incubator and inoculating wire.

Method

- 1 5 ml sterile MR VP broth was taken in a test tube.
- Sample to be tested was inoculated with the help of sterile inoculating wire into the broth.
- It was incubated at 37^{0}C for 24-48 hours.
- 0.6 ml alpha- Naphthol/ml of broth was added and then 0.2 ml 40% KOH/ml broth was also added.
- The color change of broth was examined after 15-20 minutes. There was pink color in positive sample and in negative cases there was no color change. *E. coli* were found as VP negative.

3.5.7 Citrate Utilization Test

Method

- Simmons Citrate media was made as described by manufacturer's instruction.
- The sample to be tested was stabbed in the medium.
- It was incubated at 37°C for 24hours.
- E. coli showed citrate negative (no blue slant)

3.5.9 Triple sugar iron (TSI) agar slant

Method

- TSI slant was prepared as described by manufacturer's instruction
- The sample to be tested was stabbed in the medium as in citrate and incubated in 370C for 24 hours.

4. Results

The questionnaire survey revealed that 62% of the farmers use water from tube wells followed by 21% use of well water and 17% of the water source was boring water. The use of boring water was found to be practiced at large scale poultry farms.

Bacteriological examination revealed presence of bacterial contaminants in 65% of the total water samples. It was found in 91% well water samples followed by, 71% tube well water and and 11% boring water sample. The result of bacteriological examination has been presented in table1.

Table 1 : Result of bacterial examination of water samples

S.N.	Source	No. of sample	+ve sample	% of +ve sample
1	Boring	18	2	11
2	Tube well	65	46	71
3	Well	22	20	91
	Total	**105**	**68**	**64.78**

Similarly, of the total water samples postitive (68) for bacterial contaminants, twenty-two samples (32.35%) showed the presence of Gram positive bacteria and 38 (55.89%) for Gram negative bacteria whereas 8 (11.76%) were found to contain mixed population of bacterial contaminants. The description of various bacterial isolates has been presented in table 2.

Table 2 : Description of various bacterial isolates

S.N.	Description	No. of +ve sample	Percentage
1	Gram+ve organism	22	32.35
2	Gram-ve organism	38	55.89
3	Mixed organism. (Gm+ve & Gm-ve)	8	11.76
	Total	**68**	**100**

It was found from the study that pH of water ranged from 6.0 to 8.0. About 2.86%, 25.71%, 42.86%, 22.86% and 5.71% of the water sample were having pH of 6.0, 6.5, 7.0, 7.5, and 8.0 respectively. The pH values of water samples have been present in table 3.

Table 3 : pH values of water samples

S.N.	pH range	No. of sample	Percentage
1	6.0	3	2.86
2	6.5	27	25.71
3	7.0	45	42.86
4	7.5	24	22.86
5	8.0	6	5.71
	Total	**105**	**100**

Out of 38 Gm –ve sample preserved in nutrient broth with 20% glycerin further biochemical tests were performed to identify *E coli*. Out of them only 8 sample (7.6%) were identified as *E coli* and other were other member of Gm-ve bacteria such as *Pseudomonas, salmonella, cholera, Klebsiella, Shigella*, etc. The result of biochemical test has been presented in table 4.

Table 4 : Result of biochemical tests

Tests	Reaction of E. coli	Tests	Reaction of E coli
Gm staining	-ve rods	Catalase	+ve
oxidase	-ve	motility	+ve
MR	+ve	VP	-ve
indole	+ve	TSI	y/y H2S –ve
citrate	-ve		

5. Discussion

From the study it was found that most of the small scale farmers in the western Chitwan use tube well (62%) water followed by wells (21%) and some of the large scale farms use boring water (17%). In general, it was found that chances of contamination are highest in well as it is an open source. From the study it was found that boring waters are safe and free of bacterial contamination. In the study, 11% of boring source were found to be contaminated but this may be due to sampling error i.e. sample taken from preserved tank. Sample directly taken from source were free of contamination.

The present study result suggests that 64.77% of total sources of water used for drinking to poultry is contaminated which is slightly higher than that mentioned by Basnet *et al* (1997). This may be due to research period in rainy season and time factor. Similar type of study in Kathmandu valley by Joshi *et al* (2004) revealed that 71.43% sources of poultry drinking water were contaminated with Coliform bacteria and *E. coli*, which is higher than this study. It may be due to geographical difference of these places.

The prevalence rate of *E. coli* in drinking water sample of poultry in western Chitwan district was found as 7.61%. From the study it was found that 2.86%, 25.71%, 42.86%, 22.86%, 5.71% of waters were examined with pH of 6.0, 6.5, 7.0, 7.5, and 8.0 respectively. The change in pH of water may be due to different causes such as chemical contamination, bacterial contamination, presence of mineral salts, etc which can cause source of problem to poultry.

6. Conclusion

From this study, it was found that prevalence of *E. coli* in drinking water of poultry of western Chitwan was 7.61%. The study also suggests that boring water source was the best source of water for poultry. Contamination of tube well was 71% and those of well was 91%. We can deduce that well water should not be used for poultry as it bears higher percentage of contamination. Farmers should be conscious about their water source contamination and regular lab tests should be carried out. The variation in pH of water from 6.0 to 8.0 may also lead to problem to the birds.

7. Recommendations

Efforts should be done to prevent pollution of the source. Following recommendation should be considered to maintain water quality.

- Frequent sanitary survey should be done to locate and identify health problem of poultry farm.
- Records of lab examination should be maintained regularly.
- Well should be dug in elevated land or should be away from water lodging area.
- Well should not be left open, it should be preferably covered.
- The source of water should be examined following the change of season.
- Routine water analysis is required to determine if water treatment procedures are necessary.
- Drinkers and waterers should be properly cleaned each time before providing water to the birds.
- Strict biosecurity should be maintained in the farm.
- In case of automatic water supply, water pipe should be cleaned with high pressure flushing which helps to dislodge the organic build up, which is ideal breeding for bacteria. Hydrogen peroxide based cleaners have proved to be highly effective in eliminating organic build up in water pipes.
- Water preservation tank should be regularly cleaned.
- Water sanitizers like Sokrena WS (1ml/5lit. of water) or bleaching powder (10gm/1 lit) should be used.
- People using same source for drinking purpose should boil or treat water before consumption.

References

Basnet H. B., D.R. Bhandari, and D.R. Shrestha,1997, Examination of Drinking

Water used for Poultry in Western Chitwan, Journal Institute of Agriculture and Animal Science 17-18 :83-86.

Chakabarti, A. 2003. A Text Book of Preventive Veterinary Medicine. Kalyani Publishers, India.

Dhakal I. P. 2002. Common Poultry Diseases and their Management in Nepal. The Blue Cross Annual Bulletin Fifth Volume. Published by NVSA.

Holt J. G. *et al*, 2000, Bergey's Manual of Determinative Bacteriology, 9th edi, Lippincott Williams & Wilkins.

Sainsbury D 2002, Poultry Health and Management, ELST Publishing.

Sharma S. N., S. C. Adlakha, 2004, Textbook of Veterinary Microbiology, Vikas publishing House Pvt Ltd.

Mishra A., R. Sharda, D. Chhabra, and M.N. Moghe, 2002, Escherichia coli isolates from domestic poultry, Indian Journal of Animal Sciences 72(9) : 727-729.

Swaminathan T. R., N. Daniel Joy Chandran, and N Dorairajan, 2004, Virulence attributes of E. coli associated with colisepticaemic chickens, Indian Journal of Animal Sciences 74(3) : 248-252.

Neupane T. R., D. R. Chapagai, B. R. Baral, 2005, Prevalence of Colibacillosis in Commercial Poultry and Antibiogram of E. coli Isolates in Chitwan District, National Poultry Expo-2005/ Nepal, Dec.15-19, 2005, Narayangarh, Chitwan.

Dhakal I. P., R. P. Poudel, N. Parajuli, 2002, Occurrence of Poultry Diseases in Chitwan Valley of Nepal, Bulletin of Veterinary Science & Animal Husbendry, Nepal, Nepalese Vet. Journal 26 :27-35, NVA.

Outbreak of parasitic gastroenteritis in goats under sedentary management in a low hill village of western Nepal- A case report

Dr. Shiv Prasad Devkota
Veterinary Officer
Dr. Vijay Chandra Jha
Senior Veterinary Officer
Regional Veterinary Laboratory, Pokhara

Introduction

Goat production is an important component of subsistence agriculture system in Nepal and is raised by all communities and classes of Nepalese society for their diverse use as meat, manure, hair and hide but also as a source of cash during emergencies. The estimated goat population of Nepal has been estimated to be 6.97 million and its meat production has been estimated to be about 39664 m in the year 2002/03 (CBS 2004) worth about 134 million US$ at the current market and exchange rates (Rs. 250/Kg of meat, 1 US$ = 74 NCR). However, the national production is unable to fulfill the need of the country, hence; a significant number of the goats are imported from neighboring countries. The economic value of sheep and goat manure has been estimated to about 4 million US$ at 1992 prices (Ghimire 1992).

The goat production system in the country is mostly traditional type and the loss in the production system is high to affect productivity. In general, high mortality, poor kid growth, delayed puberty, higher interkidding interval are the major factors affecting productivity. In the mid to lower hills goats are raised under sedentary management system in smaller flocks of 10-50 animals. In some village where cropping intensity is high, pasture and labor availability are lower and flock sizes is even smaller, and goats are raised under stall fed management. In the sedentary system, animals graze continuously over limited grazing land during the day and are housed indoors during night. The grazing area is usually private crop harvesting in winter, grazing is mainly on the follow fields.

Among the various factors affecting productivity, gastrointestinal nematode infection has been regarded as one of the important cause (Shrestha 1994). Thakuri and Mahato (1990) similarly stated that gastrointestinal nematode infection in goats in the eastern hills is recognized to be a serious parasitic problem which causes significant problem to the development of goat farming. Likewise, Karki (1987) reported that GI nematodes are regarded to be the most important disease problem in both the management system i.e. Migratory or sedentary system. Joshi (1991, 1994) recorded high mortality due to gastrointestinal nematode infection in sheep and goats under intensive grazing managements in mid and low hilly regions of west Nepal and further stated that parasitic gastroenteritis is one of the major causes of productivity loss in goats in Nepal. Joshi (1996) found that sub clinical parasitism was common in goats raised either under the sedentary or migratory management and further stated that the infection was responsible for the reduction in body weight gain by 93 to160 percentage. The community grazing areas were the

source of infection for the sedentary animals (Joshi 1996) and further said that the main period of pasture infection was confined to the wet summer months between Aprils to October with a very low level of infection during the rest of the year. *Trichostrongylus spp* were the most prevalent species followed by *Ostertagia spp*, in the migratory and *Haemonchus contortus* in the sedentary animals. It has been suggested that in dairy goats, a faecal egg count of >2000 EPG would be indicative of clinical disease and the faecal egg count of 500-2000 could be considered for sub-clinical parasitism (Lloyd, 1987 cited by Joshi 1994).

Case history

In October 18, 2005 a president of Suraundi Commercial Goat production Group from Pauwegaunde VDC – 8, Syanga District, complained the death of eight goats (three adult and five young) in the village among 250 goats of Khari breed during one month period and about six to eight goats were still sick. The major symptoms in the affected and dead goats as reported by the farmer were diarrhea, posterior paralysis and anorexia. A team from the RVL, Pokhara visited the site to investigate the problem and visited the site. The symptoms recorded in the field were diarrhea in some goats and some had constipation, loss of appetite, inability to move, general weakness, loss of condition, body temperature of 102^{0}F, anemic mucus membrane without a posterior paralysis of limb. All these symptoms suggested for the gastrointestinal parasitism. We collected faecal samples in plastic bags with formalin swab for EPG counts and without formalin swab for larvae culture. Along with a faecal sample, we collected blood samples from six severely affected goats for laboratory examination. All these sick goats were given Ivermectin Injection at the dose rate of 1 ml per 50 kg body weight basis on the same day and advise the farmers to drench all other goats against the nematode.

The faecal samples collected from the sick goats were examined by improved modified McMaster Method (MAFF, 1986) as stated below.

1. Three grams of faecal material was weighed and placed on the mortar and about 42 ml ofwater was added to it.
2. The mixture was gently grinded using a pistil until the faecal material was uniformly broken down.
3. The mixture was poured through the wire mesh screen and caught the strained fluid in a bowl. The debris left on the screen was discarded.
4. About 15 ml of the solution of the stained fluid after mixing regularly was drawn on the centrifuge tube and subjected to centrifugation for about 2 minutes at 1500 rpm.
5. The supernatant was discarded and the sediment was agitated until loosened and form a homogenous mixture and mixed the saturated salt solution to it and further agitated with a wooden stick.
6. Now the solution was kept for 2-3 minutes and the fluid was drawn in a sufficient amount with a pasture pipette and carefully ran into one counting chamber of McMaster slide for counting the eggs.
7. The number of eggs counted inside the counting chamber was multiplied by 100 to get the EPG count.

The faecal samples collected without formalin was processed for the larvae identification as follows.

1. About 10 grams of faecal material was weighed and poured on a mortar and same amount of wood dust was added to it and mixed using pistil after sufficient amount of water to make the content moist.
2. The prepared content was poured on the beaker and left at room temperature for about 10-14 days. The moisture content was maintained daily by adding required amount of water to it but not making the content very wet.
3. After 14 days the beaker was fully filled with water and inverted on a Petri plate by slightly tilting the Petri plate.
4. After 4-5 hours the larvae migrated were come out to the water on the Petri plate. Thus the fluid on the plate was drawn using a pasture pipette and collected in a test tube. The centrifuge tube after labeling was kept in a refrigerator for about a night.
5. Next day the supernatant was discarded and the sediment where larvae was deposited was mixed with a 2-3 drops of Lugol's iodine and transferred to the slide and viewed on a microscope using x10 objective lens and counting of the larvae and their identification was carried out on their morphological characteristics.

Findings

As the goats used to graze on the nearby marginal land throughout the rainy season and on the fallow rice fields after harvesting the paddy, all the goats were heavily infested by the gastrointestinal helminthes. The result of the faecal examination for Strongyles eggs and the mean faecal larval composition are presented in the table 1 and 2 respectively.

Table 1 : Faecal Egg Count of Goats

Number of animals	EPG of faeces		
	Before Treatment		After Treatment
	Mean EPG	Range of EPG	
6	9060±5466.90	4100-18600	0

Table 2 : Mean Fecal larval composition of the faecal material examined after larval culture

Nematodes species	Mean larvae count	Percentage of mean larvae recovered in the faeces
Trichostrongylus spp.	22	28.95
Ostertegia spp	13	17.10
Oesophagostomum spp	7	9.21
Haemonchus spp	32	42.16
Nematodirus spp	2	2.60
Total	**76**	**100**

Discussion

Considering the clinical findings, seasons, grazing lands, faecal examination and treatment response in the sick animals and the population, it can be stated that parasitic gastroenteritis (PGE) was the cause of death in the goats. Though faecal egg count has been regarded as a crude estimate of the worm burden, it has been suggested (Lloyd 1987 cited by Joshi in 1994) that in dairy goats, a faecal egg count of >2000 EPG would be indicative of clinical disease and the faecal egg count of 500-2000 could be considered for sub clinical parasitism. In this flock, all the sick animals had faecal egg counts of > 4000 indicating that these animals were suffering from severe clinical helminthes infection. Thompson (1990) suggested that as few as 1000 *H. contortus* can cause clinical anemia and hypoproteinaemia in young goats and about 2500 *H. contortus* can cause death.

Though a mixed infection was found in the faecal culture, it can be said that *H. contortus* and *Trichostrongylus* spp were the main worm species responsible for the mortality in the flock. *H.contortus* parasite was also reported as a major cause of death in goats in Malaysia (Sani *et al.* 1985 as cited by Joshi 1994). Baxendell (1987) cited by Joshi (1994) also stated that severe haemonchosis in goats occurred when EPG exceeded 2000. Due to high fecundity and short generation interval under optimal environmental conditions, development of this parasite is very rapid and thus animal become severely infected. Mortality of some 8 goats in the flock in this case is not unusual in severe haemonchosis.

The other gastrointestinal helminthes found in the faecal larval culture were *Trichostrongylus* spp., *Ostertagia* spp., *Oesophagostomum* spp and *Nematodirus* spp. The effects of these parasites, as reviewed by Soulsby (1982), could be summarized as loss of appetite, villous atrophy, reduced intestinal efficacy. These parasites certainly would have contributed to the gradual debility of the animals.

In conclusion, it can be said that, mortality and other productivity losses due to parasitic gastroenteritis can be severe in goats even under small holder sedentary management systems. From the present observations it can be said that if goats are grazed on limited pasture and continuous grazing system heavy morbidity and mortality would result due to helminthes infection and a regular anathelmintics treatment with a broad spectrum anthelmintics becomes necessary.

References

Joshi, B R (1991). The effect of parasitic gastroenteritis on sheep productivity under intensive grazing management in the mid-hill region of Nepal. Paper presented at 4th Indian National Congress of Veterinary Parasitology, Anand, India, 22-24, November, 1991

Joshi, B R (1994). Effect of parasitic gastroenteritis (PGE) in goats under sedentary management in a low hill village of Western Nepal. A Clinical Report. Veterinary Review. 9(1) 18-20, Pakhribas Agriculture Centre, Dhankuta, Nepal.

Joshi, B R (1996). The need and strategies for gastrointestinal Nematode Control in the sheep and goat population of Nepal. Bull.Vet.Sc. & A.H. Nepal 24 :59-70

Thakuri, K and Mahato, S N (1990). Prevalence of gastrointestinal helminthes infections in ruminant in Dhankuta District. In : Livestock in the hills of Nepal-2, (Gatenby R M; Thapa, B and Shresth, N P eds). Proceedings of Second Livestock Workshop held at Pakhribas Agriculture Center, Dhankuta, 11-16 March, 1990.

Karki, N P S (1987). Sheep resources in Nepal and some constraints in migratory system of production. Paper presented at Second National Conference of Nepal veterinary Association, 23-25, Feb., 1987.

Ghimire, S C (1992). The role of Small Ruminants. In : Sustainable Agro-ecosystem of Nepal (Eds : Abington, J.B.), FAO Animal Production and Health Paper No. 105, Rome, pp : 77-109.

CBS (2004). Central Beuro of Statistics. Government of Nepal, Kathmandu

Thompson, K G (1990). Gastrointestinal diseases of goats. Goat Health and Production, refresher course for veterinarian, June 11-15. University of Sydney Proceedings 134, pp : 253-264.

Soulsby, E J L (1982). Gastrointestinal nematode infection in ruminants. In : Helminthes, arthropods and protozoa of domestic animals. Seventh edition. Bailliere Tindall, London.

Investigation of bovine reproductive disorders in cows and buffaloes in western region

Dr. Vijay Chandra Jha
Senior Veterinary Officer
Dr. Shiv Prasad Devkota
Veterinary Officer
Regional veterinary Laboratory, Pokhara

Introduction

In Nepal infertility problem in crossbred and exotic cattle has been reported to be the most prioritized problem in dairy pocket areas in the country (Jha, 2000). A study conducted in Kathmandu valley in improved cattle revealed that among the various reproductive disorders; anoestrus repeat breeding and abortion was 45%, 27% and 5% respectively (Khanal, 1996). Reproductive disorders such as anoestrus and repeat breeding were reported to be 21% and 36% respectively in breedable improved cows and heifers in Pokhara valley (Sankhi, 1999).

Infertility in animals is associated with microbial pathogens, anatomical abnormalities, hormonal imbalance, nutritional deficiency, hereditary defects and extreme climatic conditions. Jha (2005) reported that out of the 118 serum samples of repeat breeder and aborted cows examined, 0.8% samples were positive for the presence of antibody of brucellosis, 9.3% for Leptospirosis and 50.8% for infectious bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis and no any sample positive for Chlamydiosis.

This study attempts to know the extent of infertility problem and to isolate, to identify the specific and nonspecific agents causing infertility in cattle and buffaloes in western region. The treatment responses of nutritional supplementation and/or antiseptic and antibiotics in infertility cases have also been evaluated.

Materials and Methods

In a close collaboration with Livestock Services Offices of Rupendehi, Kaski, Syanja, Gulmi, Palpa and Tanahun districts; the infertility camps were attended and also the dairy farms having cows and buffaloes with infertility problems were visited. Clinical examination and rectal palpation were performed and where appropriate pathological samples were collected for laboratory investigation.

Twelve vaginal swabs and 52 uterine mucus samples were collected from the cows and buffaloes which had pathological condition of Endometritis and /or Vaginitis and/or Cervicitis with a history of repeat breeding and/or abortion. The vaginal and uterine mucus samples were preserved in the Cary Blair transport media until the samples were subjected for Campylobacter and other bacterial examination in the laboratory. For Trichomonads the cervical and/or uterine

mucus samples were examined on a slide under a microscope. Blood samples were collected for serological examination of brucellosis.

At the time of visit to the farms, immediately after clinical examination some of the cows were found having inactive ovaries or Endometritis and/or Cervicitis were treated with following therapies.

Group-1 : Anoestrus

- Vitamin ADBE injection 5 ml I/M on alternate days (Total two doses)
- Minerals powder 50 gm/ animal/day orally for 10 days.
- Tonophosphon injection 10 ml I/M on alternate days (Total two doses)
- After above treatment Janova 3 capsules on 11th day and 3 capsules on 12th day were given orally.

Group-2 : Repeat breeder

- Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine.
- Gentamycin injection 25 ml I/M daily for three to four days.
- Minerals powder 50 gm/ animal/day orally for 10 days

Group-3 : Abortion

Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine.

Tetramycin LA injection 20 ml I/M on alternate days (total 3 injections)

The concerned farmers were advised to provide 200 gm of mustard cake with the concentrate feeding to the cows and buffaloes whose body condition was not so satisfactory

After 5 months a follow up visit was made to know the reproductive status of the treated cows and buffaloes.

Results and Discussion

The reproductive problems recorded among the breed type are presented in table 1. The Disease condition found during rectal examination of cows and buffaloes are presented in table 2.

Table 1 : Reproductive problems recorded among the breed type

Species/Breed	Age group (Years)	No of cows with reproductive problems examined		
		Anoestrus	Repeat breeding	Abortion
Cow/Jersey	2-10 yrs.	26	32	10
Cow/Holstein	3-13 yrs.	5	12	2
Buffalo/Murrah	3-10 yrs.	9	7	2
Buffalo/local	2-7 yrs.	2	6	5
Total	**118**	**42 (35.6%)**	**57 (48.3%)**	**19 (16.1%)**

Table 2 : Disease condition found during rectal examination of cows

Condition	Endometritis /Pyometra	Cervicitis	Vaginitis /Vulvitis	Inactive ovary	Follicular cyst	Luteal cyst	No any disease condition
Anoestrus	-	8	5	21	-	-	8
Repeat breeding	9	20	7	-	10	3	10
Abortion	2	4		-	-	-	11
Total	**11 (9.3%)**	**32(27.2%)**	**12(10.2%)**	**21(17.8%)**	**10(8.5%)**	**3(2.5%)**	**29(24.5%)**

Table 3 : Bacterial species isolated from the uterine mucus samples

Bacterial species	No. of isolates
Bacillus species	41
Escherichia coli	24
Staphylococcus species	17
Streptococcus species	10
Micrococcus species	2
Enterobacter species	2
Proteus species	9
Pseudomonas species	7
Total isolates	**112**

It can be seen in table 3 that the most common isolates from the vaginal and uterine mucus were *Bacillus species* followed by *Escherichia coli* and *Staphylococcus species*. The pattern of bacterial isolates found in this study indicates that most of the isolates, which does not seem to be pathogenic in healthy condition, may be pathogenic when the reproductive organs are under stress or injured Singh *et al* (1996) reported that the *Bacillus spp.* was more frequently isolated followed by *Escherichia coli* and *Staphylococcus aureus* from cervical-vaginal mucus of repeat breeder cows. Deshmukh and Markandeya (1995) reported that ascending types of microbial infections and residual micro flora of genital tract of cows under certain conditions lead to low grade Endometritis resulting into repeat breeding.

Among the isolates examined for antibiotic sensitivity tests, most of the isolates were susceptible to gentamycin, chloramphenicol, enrofloxacin and cefotaxime. Majorities of the tested isolates were found to be resistant to amoxicillin, cotrimoxazole, oxytetracyclin and cloxacillin as shown in table 4. Based on this finding the gentamycin, chloramphenicol, enrofloxacin and cefotaxime can be promising drugs to treat the infectious cases of infertility caused due to bacterial infections.

Table 4 : Antibiotic sensitivity test of some bacterial isolates

Isolate	No. of isolates tested	AM	EX	G	CF	CX	CO	C	K	O
Escherichia coli	24	5	24	22	22	6	4	19	20	5
Pseudomonas spp.	7	4	7	6	6	0	0	5	5	0

Staphylococcus spp	17	4	16	16	15	1	0	13	13	1
Bacillus spp	41	2	40	39	39	0	0	33	34	3
Streptococcus spp.	10	8	10	8	8	7	2	6	7	0
Micrococcus spp	2	1	2	2	2	0	0	2	2	0
Proteus spp	9	1	9	9	8	0	0	6	6	1
Enterobacter spp	2	1	2	2	2	0	1	1	2	1

Note :AM = Amoxicillin, Ex = Enrofloxacin, G = Gentamycin, CF= Cefotaxime CX = Cloxacillin CO= Cotrimoxazole C=Chloramphenicol, K= Kanamycin, O = Oxytetracycline.

The cervical and uterine samples tested for Trichomonads microscopically, none of the sample was found positive. The cervical and uterine samples subjected for campylobacter isolation were found negative. The 33 serum samples subjected to the Rose Bengal Plate Test for the detection of antibodies of Brucella were all negative.

Table 5 : Treatment response in cows and buffaloes with reproductive disorders

	Treatment regimen	No. of animals treated	No. of animals followed up after 5 months	No. of animal pregnant
Group-1 Anoestrus	Vitamin AD3E injection 5 ml I/M on alternate days (Total two doses) Minerals powder 50 gm/ animal/day orally for 10 days. Tonophosphon injection 10 ml I/M on alternate days (Total two doses) After above treatment Janova 3 capsules on 11th day and 3 capsules on 12th day given orally.	42	28	18 (64.3%)
Group-2 Repeat breeder	Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine. Gentamycin injection 25 ml I/M daily for three to four days. Minerals powder 50 gm/ animal/day orally for 10 days	57	38	22 (58%)
Group-3 Abortion	Povidone iodine solution 20 ml mixed with 10ml normal saline administered intrauterine. Terramycin LA injection 20 ml I/M on alternate days (total 3 injections) After 3 to 4 months a follow up visit was made to know the reproductive status of the treated cows and buffaloes.	15	10	7 (70%)
	Total	**114**	**76**	**47 (61.8%)**

It can be seen in table 5 that out of 114 infertility cases treated; only 76 treated cases could be followed up after 5 months. Out of 76 followed up cases 47 (61.8%) cases were found to be pregnant. The treatment response of the cows and buffaloes was found promising. Therefore the treatment therapy applied in this study can be a promising therapy for the treatment of cases with infectious form of infertility particularly infertility due to microbial origin. Further future works in detail needs to be done on the identification of various causes of infertility including infectious causes and development of its strategic control measures.

References

Deshmukh, VV and Markandeya, NM (1995) A study on antibiotic sensitivity pattern of bacterial isolates from repeat breeding cows, Int. J. Anim. Sci. 10 : 337-338

Jha, VC (2000) Study on infectious causes of infertility in crossbred & exotic cattle in Nepal. Annual Report 1999/2000, Animal Health Research Division, Tripureswor : 19-23.

Jha, V C (2005) Study on infectious causes of infertilityand its management in crossbred & exotic cattle in Nepal.Nepalese Veterinary Journal, Vol. 28 : 25-31

Khanal, DR (1996) Proceedings of First National Workshop on Livestock and Fisheries Research in Nepal, May 7-9, Khumaltar, Lalitpur.

Sankhi, K.P. (1999) Souvenir, Sixth National Veterinary Conference, Nepal.

Singh, N.P. Chaturvedi, V.K. and Singh, DP (1996) Bacteriological studies on repeat breeder bovines, Indian Vet. J. 73 : 462-463.

Kumri in goat : An outbreak investigation in Banke district of mid-western region of Nepal

Dr. Kedar Karki
Veterinary Officer
Central Veterinary Laboratory

Abstract

Seasonal occurrence (mainly in October-November) of a disease syndrome locally called 'Kumri' meaning weak back was observed in goats in Banke and other districts of western Terai in the last few years. Traumatic injury to the lumbar region, nutritional deficiencies and parasitism in the spinal cord were the likely causes considered. Based on the epidemiological pattern viz; seasonal occurrence, clinical symptoms, afebrile condition and local nature of infection, and non response to supplementation of vitamins and minerals, the disease was provisionally diagnosed as cerebrospinal nematodiasis. This has been further substantiated through laboraotry of Seteria spp in cattle in this region, detection of microfilaria in affected goat and treatment response of affected goats with diethylcarbamazine. As adult seteria spp in cattle, Buffalo and microfileria from blood smears of affected goat confirmed the cerebrospinal nematodisease in goat in Nepal.

Introduction

There was an outbreak of peculiar syndrome in goats in Kusum, Mahadevpuri, Kamdi, Kohalpur, Kachnapur Village Development Comities of Banke district of west region of Nepal was observed during October/November 2006.In this V.D.C. out of total population of 7434 Goats 2028 were affected by this syndrome when treated with diethylcarbamezine(Hetrazan) 1866 goats recovered and 162 died. During outbreak goats above twelve months of age were mostly affected. Typical clinical signs in affected goats were paralysis of one or both fore/ hind limbs, paralysis of Lumber region in coordination and swaying back gait. When hand fed affected animal seat normally and there was no systematic involvement,(no rise in Temperature diarrhea).Since year 1986-1987(Karki) reported same pattern of disease in this area with morbidity 25.30% and mortality 12-15% were recorded. When these animals were treated with diethylecarbamzan 10mg/kg disease entity started to disappear within 5-7 days, but there was 2-5% post recovery.

Review of Litrature

Goats are considered as hardy and resistant to many infectious diseases but parasitic diseases in them are considered to be major cause of considerable economic loss, which arise primarily from the failure of goats to grow or perform satisfactorily. Several species of parasites are involved and the relative importance of species in a particular region varies with its agro-climatic and husbandry practices.

Posterior paralysis (Kumri) in goat is considerd to be caused by a filarial parasite; *Setaria* spp. *Setaria labiatopapillosa* (synonym : *Setaria digita, Setaria cervi*) normally occurs in the peritoneal cavity of cattle, buffaloes and deer. The parasite in the peritoneal cavity of these animals is not generally pathogenic. However, the immature forms in non-natural hosts like sheep and goats cause cerebrospinalnematodiasis (posterior paralysis, kumri) with different neurological signs which is often fetal. During 1986-87, there was an outbreak of peculiar syndrome in goats in Banke district of west region of Nepal during October/November. Goats above six months of age were mostly affected. Typical clinical signs in affected goats were paralysis of one or both hind limbs, paralysis of lumber region, incoordination and swey back gait. When hand fed, affected goats sit normally and there was no systematic involvement, no rise in temperature and diarrhea with morbidity 15.20% and mortality 2-15% were recorded. On treatment with diethylecarbamzan 10mg/kg affected animal disease entity disappear with 5-7 days. But there was 2-5% post recovery deformity was recorded (Karki 1996).

The meningial worm (*Parlaphostrongyle tenius*) also known as the deer worm due to its aberrant migration in sheeps and goats causes damage to central nervous system with clinical signs ataxia, stiffness, muscular weakness posterior paresis, paralysis, head tilt arching back. Clinical sings generally begin in the hind limbs and progress to front limbs (David E Anderson, 2002).There was consistent abnormally shift in nucleated cell count from predomently lymphocytes and monocytes to eosinophils over the course of infection. *Parelaphostrongylus tenuis* is normally found in the venous sinuses and subdural space of the brain of white tailed deer in eastern northern America. Moos caribou, reindeer, sheep, goat are susceptible to infection. However they are abnormal hosts in them it causes cerebrospinalnematodiasis, a disease of nervous system, often resulting to death (DNR-Brain worm2001-2006. Sheep and goats are considered dead end host of meningeal worm once the parasite if ingested by sheep or goat, it may migrate through different part of body wrecking havoc with the animal (J.S. Rook et.al.). Sheep and goat are considered dead –end hosts for *P. tenuis*. The neurological sings observed in infected sheep, goat depend upon the number of larvae present in nervous tissue and specific portion of brain or spinal cord, a mild infestation in a local area may produce slight limp, or weakness in one or more legs. A more sever infestation may cause animal to become partially or completely paralyzed (M. Kopcha *et al*, Susan Schoenian, 2005, SCWDS Briefs, 1992, Corry Jeanne Mortensen, 2000, Pusterla *et al*, 1997, Kopcha M 1989, and (F S Guthery *et al* 1979).

Setaria digitata and *S.marshali* larvae were observed in cerebrospinal cavity of two paralyzed cattle in Taiwan. Affected cattle showed quadriplegia and lumbar paralysis (Kwong-Chung Tung *et al*, 2003), El-Azazy O.M.E. 1999, recorded patent *Setaria digitata* in five out of 48 goats in Saudi Arabia. Subhachalat P *et al*, 1999 identified the worm morphologically collected from Thai cattle. Karki *et al*, 2000 detected male, female adult *Setaria* parasite from peritoneal cavity of zebu cattle and buffalo during post-mortem examination in Banke.

Mukhopadhyay S *et al*, 1996 implanted adult gravid female of bovine filarial worm in Mastomys coucha found microfilaraemia which was detected as early as 4 days post plantation. Implantation resulted in a decrease in total leuckocytes and erythrocytes and induction of eosinophilia.The microfilaria in circulation were found to be eliminated by oral administration of diethylcarbamezine citrate, indicating its usefulness as potent anti-micro filarial drugs. There was

slight eosinophila in affected goat (S.P. Shrestha). Prevalence of Lumber paralysis caused by cerebrospinal nematodiasis is common in goats all over India mainly during the month of October-December with morbidity as high as 31%. Prophylatic treatment with Hetrazen (diethylcarbamazine at the onset of winter is highly effective for control of lumber paralysis in goat (P. Ghalsasi *et al*, 2000).

Objectives

- Haematological investigation for detection of Microfilaria in affected goat.
- Haematological analysis of RBC, WBC.Hbg.PCV OF blood from affected goat.
- Evaluation of treatment response of Diethyl carbamezene.

Methodology

An epidemic investigation of the codition of posterioir paralysis in goat was conducted in the affected villages of mid-western development region. The detail about the site of investiongation and the number of animals affected and dead is presented in table 1.

Location	Number of goats at risk	Number of goats affected	Number of goats died
Kusum	562	175	20
Mahadebpuri	1720	480	27
Kachnapur	1552	390	35
kohalpur	1825	495	45
Kamdi	1775	498	35
Total	**7434**	**2038**	**162**

A total number of 10 blood samples were collected and made into smear. The blood smears were fixed in methanol and stained with Giemsa;s stain and examined under compound microscope. Based on the clinical manifestations, the condition was provisionally diagnosed as cerebrospinalnematodiasis locally known as Kumri and the animals were treated with Drethylecarbamezin (Hetrazen Banocide fort) in outbreak areas.

Result and Discussion

Of the 10 blood samples, six of them revealed the presence of typical microfilaria with sheath is most easily seen as it extends beyond the anterior and posterior ends of larvae. On the basis of clinico-epidemiological study, finding of adult Seteria spp in the cattle/ buffalo in areas of outbreak and also microfilaria in blood from diseased goat confirms *Setaria* spp as the main cause of posterior paralysis.The finding of haematological analysis indicated a marked decrease of totat RBC, WBC, haemoglobin content, PCV, but a marked increase in eosinophil confirm the finding of other researcher for the occurrence of filarial infestation. Response of treatment was similar to earlier worker also confirmed in this entity is caused by *Setereria* spp. The result of the study of haematological parameters is presented in table 2.

Table 2 : Result of haematological analysis of blood

Haematological parameters	Normal value (%)	Mean ± SE (%)
Basophil	0-3	0.46+ 0.10
Esionophil	1-8	8.78+ 0.38
Monocytes	1-5	0.93+ 0.16
Lymphocytes	40-75	57.63 + 1.3
Neutrophil	10-50	32.20+ 1.25
TEC	8-18 Millions/mmc	3.3-4.6Millions/mmc
TLC	13 - 15 × 10^3/mm^3	6.2 - 8.5 × 10^3/mm^3
Haemoglobin	8.8-13.8%	6.6-9.3%
PCV	25-40%	20-28%

Recommendation

On the basis of above finding, confirm the in specific Agro-ecozone in specific season, out break of posterior paralysis (Kumri) is caused by Seteria spp. and response of Diethylcarbamazin on its treatment is recommended as soon as possible. Same treatment if applied can prevent the loss.

References

A.K. Upadhyay : Setariasis, Cerbrospinalnematodiasis : Preventive Veterinary Medicine IBDCO Publishing House, First edition 2005 pp422-424

Corry Jeanne Mortension *et al;* Parrlaphostrongylus (Brainworm) Infection in Deer and Elk.Western Collage of Veterinary Medicine; http ://www.usask.ca/wcvm/herdmed/specialstock/elk/diseases/Ptenius.html

C. Devendra, G.B. Mcleroy : Goat and sheep production in the tropics, Reprint 1990. (Page 2-3)

David E Anderson 2002 : PARASITES : Parelaphostrongylusa tenius (Meningeal Worm) http ://www.vet.ohio-state.edu/docs/ClinSci/camlid/mening.html.

DNR-Brainworm Michigan.gov.Home; Michigan DNR Wildlife Disease Laboratory.

E.J.L. Soulby : Heminth Arothopodes and protozoa of Domesticated, Animals seventh Edition 1986 pp 316-3

El-Azazy O.M.E *et al* : Patent infection with Setaria digitata in goats in Saudi Arabia : Veterinary Parasitology, Vol.82, Number2, 31March 1999, pp.161-166(6).

FS.Guthy *et .al;* Cerebrospinal nematodiasis caused by Parelaphostrngylus tenius in Angora goats in Texas : Journal of Wildlife Diseases,15(1),1979, pp.37-42.

J.S.Rook, *et.al* MeningealWorms (Brain Worms) &Liver Flukes (Deer Flukes) Two Uncommon Internal Prasites

Karki Kedar and B.N. Adhikari : (Cerebrospinal nematodiasis Goats In Western Terai of Banke District- A Review) Nepalease Vet. J. 26 : 98-100 (2000)

Karki K.B.Paralysis in goat; cerebrospinal nematodiasis (in Nepali – Veterinary chaumasik, Tissue 1, 2053, B.S. pp. 25-26)

Kwong-ChungTung *et al*; Cerebrospinal setariosis with Setaria marshalli and Setaria digitata infection in cattle; J.Vet.Med Sci.2003 Sep; 65 :977-83.DOI : 10.1186/1475-2883-2-S1-S4.

Mukhopadhyay S;*et.al*;Setaria digitata microfilareamia in Mastomys coucha : an animal module for chemotherapeutic and immunobiological studies; Parasitology, 1996, vol.113, nO4, pp323-330,Cambridge University Press ,Cambridge,ROYAUME-UNI(1908)(Revue).

M.Kopcha :et.al : Cerebrospinal nematodiasis in a goat herd : J.Am.Vet. Med. Assoc. 1989 May 15; 194 :1439-42.

Mchel Boussingnesq; *et.al :* Clinical picture, epidemiology and outcome of Loa-associated serious events related to mass ivermectin treatment of oncocerciasis in Cameroon; Filaria Journal 2003, 2(Suppl 1) :S4 *DOI : 10.1186/1475-2883-2-S1-S4.*

M.Kopcha;*et.al*;P.tenuis-White-tailed Deer Parasite;MSU Extension & Ag.Experiment Station,Mechigan State University;Collage of Veterinary Medicine.

O.M. Radiostitis D.C. Blood C.C.Gay : Certebrospinal Nematodiasis Lumber Paralysis, Kumri.)Earth edition 1994 (Page No 1274-125-75)

P.Ghalsasi *et al* : A study on the prophylaxis of lumber paralysis caused by cerebrospinal nematodiasis in goats : 7th Ibternational Conference on Goats ,France,15-21 May 2000 :853.

Pusterla N : *et.al* : Cerebrospinal nematodiasis in seven goats : Schweiz Arch Tierheilked.1997; 139(6) :282-7.

Statistical Information on Agriculture 1997/1998 H.M.G. Agriculture statistics Division, Nepal. 2002/2003 (Page 29)

Subhachalat P, *et.al* : Setaria digitata in cattle of Thailand identified by sodium dodecyle sulfate polyaceylamide gel electrophoresis, J.Vet.Med, Sci.1999April; 61(4); 443-5.

Setariadigitata;http;//www.nehu.ac.in/bic/HelMinth_Parasite_NE/Setaria%20digitata.html.

Susan Schoenian; Meningeal Worm, Brain Worm-Deer Worm Paralaphostrongylustenius; http ://www.sheepandgoat.com/articles/deerworm.html.

Veterinary Parasitology-Nematode Lab2 (Lungworms and Filarids www.cvm.umn.edu/ academics/course_web /current/cvm6202/Labs/lab6pdf.

Yadav C.L. Agro climatic influence on parasitic disease of sheep and goat volume 15 issue –04 (2000) pp-1.

Microbial quality of marketed pouch milk in Kathmandu valley

Dr Karuna Sharma
Veterinary Officer
Central Veterinary Laboratory

Introduction

Nepal is a least developed country with per capita income of USD 240. Agriculture is contributing about 39% to the GDP and 81% of people depend on it. Livestock sector contributes 31% to AGDP and 4% to the total export. Livestock contributes close to 50% of household income in the higher altitude 36% in mid hills and above 28% in terai (Shreastha and Sherchnad 1988).Milk has been one of the most important animal products which is evident by its sizeable sharing in the AGDP. Livestock farming is mixed farming system in Nepal, with crop production, forest and fodder management, management of communal land and draft and transportation. The percentage of animal holding records in various species is 68.2% cattle, 47.2% buffalo, 0.42% yak, 51.2% sheep, 9.74%, pigs and 47.4 poultry (CBS, 2004). The livestock populates at the rate of 4 livestock units per holding and 5 units per hectare of cultivable land. The production system is still traditional based on native breed with low productivity but well adopted in the given diversified local environment.

At present milk is collected from 43 districts and yak milk from six districts. The total production of milk was 1.1 million 96 thousand MT in 2003/2004 and targeted to reach 1.2 million 25 thousand MT by 2004/2005. The yearly milk productivity is 387 kg per cow and 830 kg per buffalo per lactation which is 10 -15 times less (for cow) than other developed countries. The growth of national milk production over the last decade is about 2.6 % per year.

Objective

To study the quality of marketed pasteurized milk in Kathmandu Valley by culture of microorganism

Materials and Methods

One hundred milk samples were collected from various dairy stalls from the market. These milk samples were collected in sterilized glass container and brought to the laboratory in chilled condition to avoid further contamination. All the milk samples were cultured in nutrient agar media at microbiology laboratory unit of Central Veterinary Laboratory, Tripureshwor to explore the presence of bacterial contaminants.

Results

Sixty one percent of total samples were found positive with the presence of *Staphylococcus* spp in 23 % of the positive milk samples, *E coli* in 16%, *Streptococcus spp in* 6 %, and Staphylococcus and *E Coli* (mixed) in 16% and 39 % of the samples were found negative for any kind of bacterial contamination. Table 1 reveals the various results of milk culture.

Table : 1 Result of the microbial culture of milk samples

S. N.	Name of dairy	Samples tested	Staph	E. coli	Strep	Staph+ EColi	-ve	% (-ve)	District
1	DDC	10	1	-	-	1	8	20%	Kathmandu
2	Sita Ram	10	1	1	-	1	7	30%	Kathmandu
3	Fresh Milk	10	3	3	-	4	-	100%	Kathmandu
4	Anmol	10	4	1	1	1	3	60%	Kathmandu
5	kalika	10	-	-	-	2	8	20%	Kathmandu
6	Sheetal	10	2	3	1	2	2	80%	Bhaktapur
7	Gayatri	10	3	-	2	3	2	80%	Bhaktapur
8	Today	10	1	5	1	-	3	70%	Lalitpur
9	Daily	10	5	2	1	1	1	90%	Lalitpur
10	Nava Prabhat	10	3	1		1	5	50%	Lalitpur
	Total	**100**	**23**	**16**	**6**	**16**	**39**	**61%**	

Discussion

The present study reveals the presence of various types of bacteria in the pocket milk marketed in the Kathmandu valley. As per international standard and food Acts, milk sould be free from any type of pathogen. Similar study results have been frequently published in national newspapers in the past which justifies the result of present study. The result of the present study also correlates with the laboratory reports of milk samples examined against mastitis at central veterinary laboratory. The bacterial population present in the mastitis milk was the highest for *Staphylococcus* spp followed by *E. coli* and *Streptococcus* spp.

Conclusion and recommendation

The present finding reveals that the milk marketed in Kathmandu valley needs be improved in quality aspect of production. Similarly, the milk pouch should be kept clean and sterile for the minimum bacterial load on the surface of the pouch so that consumers get hygienic milk which has the least population health risk. Also the existing rules and regulation need to be followed strictly so that frauds and mischief are not escaped from the arm of law.

Poultry diseases diagnosis procedure : A review

Dr. Vijay Chandra Jha
Senior Veterinary Officer
Region Veterinary Laboratory, Pokhara

The proper diagnosis of diseases depends on three important factors; identification of vital organs and body structure, knowledge of disease symptoms and lesions and a systematic plan for examining the bird's body. To begin a disease diagnosis involves the study of a series of information about the patient and its environment. It is also true for poultry disease diagnosis and it begins as follows.

Flock History

Poultry diseases must be considered as diseases of the flock rather than individual diseases. Symptoms in a few individual birds are usually an indication of a more serious flock-wide problem. It is important that an accurate flock history be recorded. The source of many diseases can be determined from this flock history. A complete flock history includes the following information

- Name and address of the owner
- Number of birds in the flock
- Breed, strain, and age of the birds

1.1 Management information

- Hatchery source
- Type of operation
- Feeding program
- A complete vaccination history

1.2 Information on the illness

- The date the illness was first observed
- Severity and number of birds affected
- Number of birds dying
- Medication history

Final remarks of disease in previous flocks and any unusual problems or conditions should be included.

1.3 External Examination

Before examining the bird internally, observe and inspect the bird for external symptoms. Note the general condition and fleshing (presence of meat on the bone) of the bird.

- Check the condition of the skin, and all natural body openings (nasal openings, mouth, ears, and vent).
- Examine the head, eyes, comb, and wattles for evidence of swelling, canker lesions, unusual discharge or coloration.
- Look for signs of lameness, paralysis, or general weakness. Inspect the affected areas for abnormalities or swelling that can give a clue to the cause. If you observe a partial or complete paralysis, note the position the bird assumes. It is often an indicator of the cause of illness.
- Inspect the bird for external parasites such as mites, lice, ticks, and fleas.

1.4 Necropsy Procedure

A necropsy is an examination of a dead animal. The only tool necessary to perform a necropsy is a sharp cutting utensil, but several good quality tools are recommended. A sharp pair of surgical type scissors and a scalpel, or knife, makes it easier to cut the necessary tissues. A pair of heavy shears helps when cutting through bones. Although few poultry disease can infect people, it is recommended that you wear disposable plastic gloves during the necropsy procedure.

Begin the necropsy by washing the dead bird with detergent water. This removes any foreign material and holds down the feathers. Place the wet bird on a flat surface with breast side up and head directed away from you.

1. Remove upper portion of the beak by cutting through the nasal cavities and turbinated bones. Turbinated bones are membrane-covered plates on the walls of the nasal chambers. This lets you observe the upper respiratory areas for the presence of infection. Squeeze the turbinate area and note if excessive matter oozes from the area. Check the eyes for inflammation (unusual reddening), mucus, or discoloration.
2. Insert one scissor blade into the mouth and cut through one corner of the mouth. Extend the cut down the neck so the interior of the gullet is exposed. Examine the mouth and larynx for abnormalities that indicate pox, mycosis, or other disease. Scan the gullet for tiny nodules (bumps) or signs of injury by foreign materials.
3. Cut the larynx and trachea from the mouth and open the trachea lengthwise. Examine its interior for excessive mucus, blood, or cheesy material.
4. Make an incision in the abdominal skin just below the tip of the breast cartilage. Extend the cut around the body on each side. Grasp the upper edge of the cut skin and bluntly peel the skin over the breast. This exposes the breast muscles. Examine them for conditioning and the presence of hemorrhages (sites of prior bleeding in the muscle).
5. Cut the skin on the abdomen where the legs join the body. Place a hand on each leg and press down and out until the femoral joints dislocate and the legs lie flat on the table. Tear the skin from the legs and check for small pin-point hemorrhages.
6. Make an incision through the abdominal muscles just below the tip of the breast bone. Do not cut too deep, or you may cut internal organs. Extend the cut toward the back and then

angle toward the point of wing attachment on each side. You must cut through the ribs in order to complete this cut. Push the breast toward the head and dislocate the shoulder joints. Cut through the shoulder joints and remove the breast from the carcass.

7. Observe the condition of the air sacs. The membranes are often cloudy and covered with mucus in diseased birds.
8. Examine the liver for unusual swelling, lesions, hemorrhages, or abnormal coloration. Make incisions into the liver and check for scar tissue and necrotic (dead) tissue. Check the spleen for hemorrhages, lesions, and swelling. Check for a cloudy, fluid-filled sac surrounding the heart.
9. Remove the liver, heart, and spleen so the digestive system is exposed. Check the digestive system for abnormal nodules, tumors, or hemorrhages. Sever the gullet near the mouth and remove the entire digestive system. You can cut the lower intestine behind the caecum for complete removal. Cut into the crop. Note if the contents are sour smelling. Wash contents from the crop and examine the lining for thickened, patch-like areas or necrotic ulcers. Check for capillary worms by making a small cut and slowly tearing the crop wall as if it were a piece of paper. Capillary worms appear as small, hair-like fibers extending across the base of the tear.
10. Open the proventriculus, the slightly enlarged area between the esophagus and gizzard, and note any hemorrhages or a white coating on the lining.
11. Open the gizzard and examine the lining for unusual roughness or lesions. Determine if the lining is separating from the underlying muscles.
12. Slit the intestine lengthwise and examine contents for the presence of worms, free blood, and excess mucus. Check the lining for inflammation, ulcers, or hemorrhagic areas. If unusual conditions exist, note in which one-third portion of the intestine the conditions are located.
13. Open the ceacum and examine the contents. Look for cheesy cores and small, ceacal worms. If you find blood, wash and examine the lining for scarring and caecal worms.
14. Check the reproductive organs (ovary and oviduct in females, testes and ductus deferens in males) for abnormalities before removing them from the body.
15. Examine the kidneys and ureter for unusual swelling or the presence of whitish salt deposits.
16. Check the sciatic nerve extending to each leg for swelling. Once you remove the kidneys, you can see this nerve as a small white fiber stretching from the spinal cord along the femur into the lower leg. Also check the brachial nerve extending from the spine, along each humerus (upper wing bone), to the wing tip.
17. Observe the lungs and bronchial tubes for lesions and unusual accumulation of mucus.

You can make notes on history, symptoms, and lesions until you are familiar enough to diagnose diseases without consulting references. It is recommended that you follow all the procedures in this publication. Often two or more diseases can infect a bird and the symptoms may be confusing. Check all affected areas before making a diagnosis and administering a treatment.

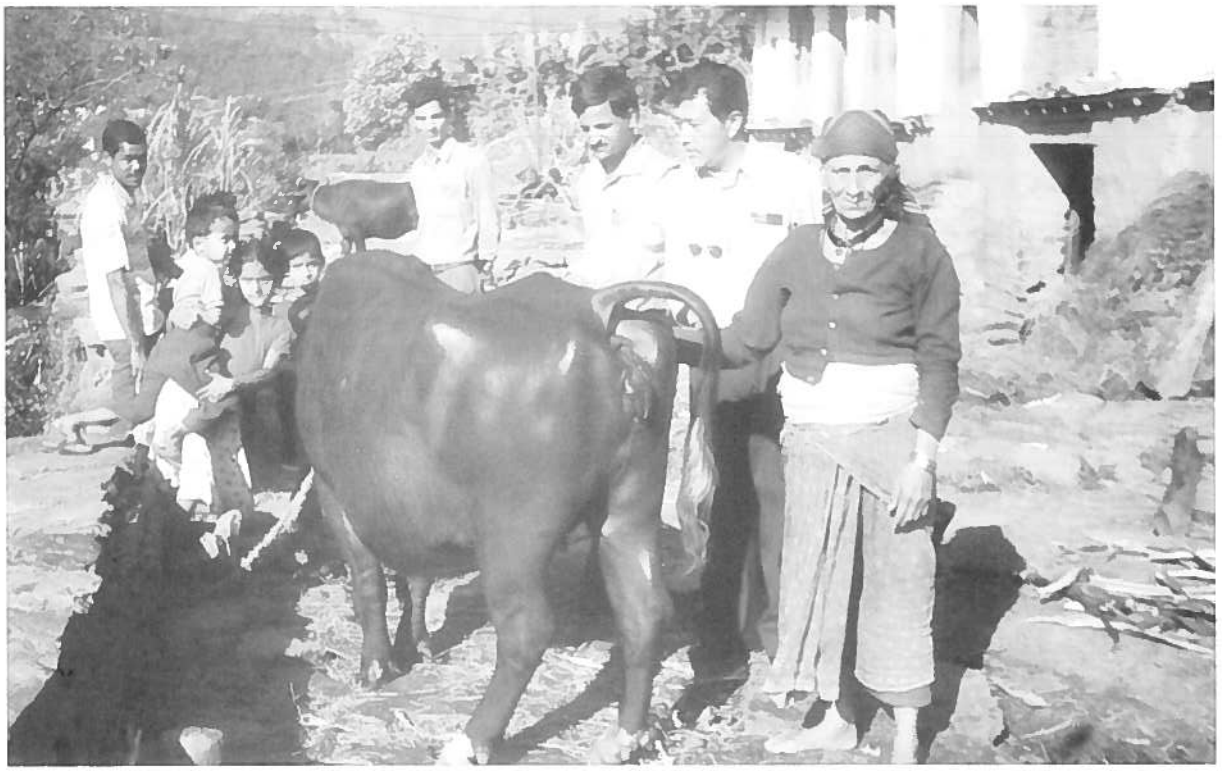


Figure 1 : Khari disease inspection in darchula

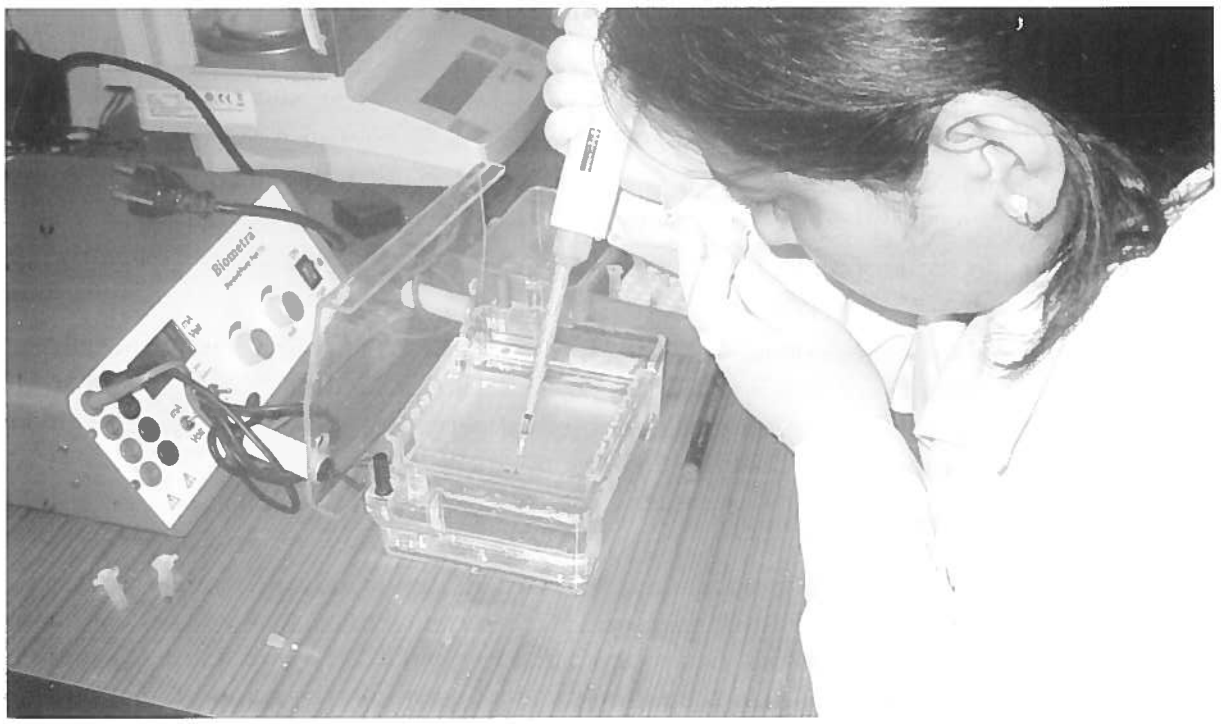


Fig 2 : PCR gel loading at CVL

Fig 3 : AI training class conducted by Australian experts (AAHL)

Fig 4 : Group photograph of tranees of AI diagnostic training

Fig 5 : Migratory birds at Taudaha, Kathmandu

Fig 6 : Post mortem examination of AI suspected birds

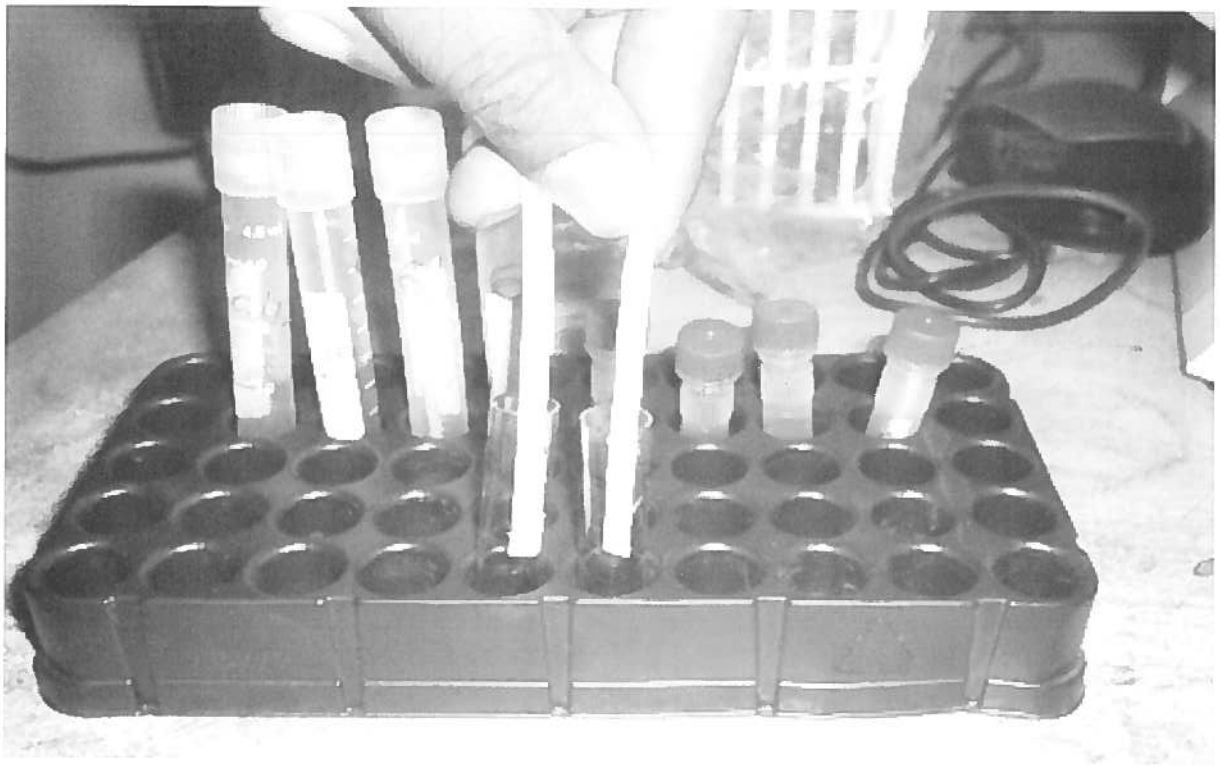


Fig 7 : AI rapid test using flu-detect kit (Synbiotic Co., USA)

Figure 8 : Participants of two weeks training on laboratory technique.